Journal of Research in Applied and Basic Medical Sciences 2024; 10(2): 154-168

Original Article

Fludrocortisone improves endometrial receptivity by regulating expression of ENaC, SGK1, HAND2, miR-200a, miR-145, miR-451, mTOR, and 4E-BP1 during the implantation window in mice

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Abstract

***Background & Aims*:** This study investigated the effect of fludrocortisone treatment on the expression of genes and proteins involved in the implantation process in mice.

***Materials & Methods*:** The study involved four groups of mice, and mRNA and protein expression were measured using real-time PCR and western blotting.

***Results*:** The results showed that fludrocortisone treatment slightly downregulated the expression of SGK1, ENaC-α, miR-145, and miR-200a, while slightly upregulating the expression of HAND2, miR-451, mTOR, and 4E-BP1 in the endometrial epithelium. mTOR kinase inhibitor PP242 treatment resulted in the upregulation of miR-145 and miR-200a, while partially downregulating the expression of p-4E-BP1, mTOR, SGK1, ENaC-α, HAND2, and miR-451 expression. Combination therapy of fludrocortisone and PP242 resulted in slightly decreased expression of ENaC, SGK1, miR-200a, miR-145, and 4E-BP1, while slightly upregulating the expression of miR-451 and HAND2 in the epithelial endometrium.

***Conclusion*:** The findings indicated that fludrocortisone did not disrupt endometrial receptivity and may even enhance it by modulating gene expression through the activation of the mTOR signaling pathway. Overall, the study suggests that fludrocortisone treatment can modulate the expression of genes and proteins involved in the implantation process in mice. The activation of the mTOR signaling pathway was also increased during the treatment. The findings indicate that fludrocortisone may increase endometrial receptivity without disrupting it, which could have implications for fertility treatment.

***Keywords*:** Fludrocortisone; Implantation, Mice, miRNAs, mTOR

Received 14 February 2024; accepted for publication 05 March 2024

Introduction

Embryo implantation requires a physiological and molecular interplay between the receptive uterus and blastocyst (1, 2). This phenomenon happens within a restricted period, allegedly known as the "window of implantation", which is defined as the time period after

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[DOI: 10.61186/rabms.10.2.154]

the ovulation process when the corpus luteum is completely made. Implantation failure is the leading cause of approximately 75% of abortion in pregnant women (3-5). Despite numerous progress made in the understanding of in vitro fertilization (IVF) and embryo transfer technology, the success rate of pregnancy through assisted reproduction technology (ART) is still unsatisfying, which is mainly due to implantation failure (6, 7).

One of the essential factors affecting the implantation process is the function of ion channels (8). Recent reports showed that the activation of the epithelial Na+ channel (ENaC) is necessary for the synthesis and release of prostaglandins, which are vital for embryo implantation (9). Furthermore, ENaC contributes to the regulation of uterine fluid absorption, which is indispensable for the closure of uterine lumen and immobilization of blastocysts (10, 11). It has been indicated that ENaC is tightly regulated by serum- and glucocorticoid-regulated kinase 1 (SGK1) (12, 13). SGK1 can enhance the ENaC expression by the suppression of the ubiquitin ligase (14). The overexpression of SGK1 could result in the increased expression of ENaC, as well as the suppression of normal implantation (15). During the window of receptivity, the expression and activity of SGK1 are temporarily diminished, facilitating embryo implantation in both human and mice (15). In addition, SGK1 could be activated via the mammalian target of rapamycin complex 2 (mTORC2) (13). The activation of mTORC2 could also phosphorylate the 4-emopamil-binding protein (p-4EBP-1) (16). It has been demonstrated that mTOR kinase inhibitor PP242, as the inhibitor of mTOR, decreases the activity of the 4E-BP1 protein (17).

Heart and neural crest derivatives-expressed protein 2 (HAND2), a transcription agent found in the uterine stroma, reported to be vital in embryo implantation in mice(18) and contributed in decidualization phase (19). It has also been shown that stromal Hand2 intervenes in implantation by reducing the differentiation of uterine epithelial cells(20).

It has recently been highlighted that besides the importance of miRNAs in cellular biology, a group of miRNAs play pivotal roles in the embryo implantation and endometrial receptivity (21). miRNAs participate as post-translational regulators of a vast majority of the biological processes including cell proliferation, apoptosis, differentiation, and oncogenesis. Several investigations reported the expression profile of miRNAs in endometrial epithelial cells (EEC) tissues and found that numerous miRNAs are aberrantly expressed in endometriosis(21). It has been implicated that the miR-200a cluster is markedly lowered during the implantation window in the murine endometrial epithelial cells (22). Recent studies showed that all members of the miRNA-200a cluster were downregulated in mice uterine between 0.5 and 4.5 days post coitum (dpc), while the markers of mesenchymal cells were provisionally upregulated (23).

MiR-145 is the maximum dysregulated miRNAs within the endometriosis patients with Recurrent Implantation Failure. It has also been shown that miR-145 is vital in the growth of the placenta in humans(24). MiR-451 has various physiological functions such as cell differentiation, cell proliferation and migration / invasion cells. All of these processes are essential for the growth and survival of endometriosis implants(25).

Various signaling proteins such as ERK, phosphatidylinositol 3-kinase (PI3K), and mammalian target of rapamycin (mTOR) contribute to the regulation and orchestration of the biological events to guarantee successful implantation (26). The mTOR protein belongs to the PI3K-related kinase superfamily, which shows a vital function in cell proliferation, growth, differentiation, and apoptosis (26, 27). The mTOR signaling pathway has a crucial role in the embryo implantation as well (28). Recent studies showed that mTOR-deficient embryos die immediately following the implantation process (29). However, the function of the mTOR pathway in the uterus during the early pregnancy period remained opaque. Some studies have indicated that glucocorticoids improve the uterine endometrial receptivity, embryo implantation, and decidualization. Fludrocortisone (FCA) is a synthetic

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[DOI: 10.61186/rabms.10.2.154]

mineralocorticoid used in conjunction with hydrocortisone to replace missing endogenous corticosteroids in patients with adrenal insufficiency. It is functionally similar to aldosterone, the body's primary endogenous mineralocorticoid, and is structurally analogous to cortisol, differing only by a fluorine atom at the 9-position of the steroid structure - this fluorination is thought to be crucial to fludrocortisone's significant mineralocorticoid potency. (30). To our knowledge, this study is the first to investigate the role of fludrocortisone in the process of implantation window, and the impact of fludrocortisone on the signaling pathways involved in the implantation and endometrial receptivity have not been well elucidated.

The goal of this investigation is to examine the effects of fludrocortisone on molecular alterations, the expression of HAND2, ENaC, and SGK1, mir-145, mir-451 and miR-200a which occur in the endometrium in response to this corticosteroid during implantation window in mice. In addition, the molecular mechanisms by which fludrocortisone acts through the mTOR-4EBP1 signaling pathway were investigated by inhibiting the mTOR signaling pathway.

Materials & Methods

Animal:

This case-control study on 40 BALB / c female mice aged 8 weeks was performed. mice were ordered from the Razi Institute (Tehran, Iran). The mice were kept at a temperature of 21 ± 1°C and humidity of 50 ± 10%, and a 12-hour light / dark period with completely free access to food and water. All animal care guidelines that were followed by Iranian Council on Animal Care guidelines. Research Ethics Committees of Tabriz University of Medical Sciences approved the experimental procedures of the current research with an ethical confirmation number: (IR.TBZMED.REC.1396.866).

Chemicals:

fludrocortisone, PP242 consumed in this research was ordered from companies (Sigma-Aldrich (St. Louis, MO)) and (Selleckchem Cat No. S2218; Houston, TX), respectively. Trizol LS reagent (Invitrogen, Carlsbad, CA) was consumed to extract RNA. Thermo Fisher Scientific kit (Cat No. EP0441; Waltham, MA) and Ampliqon Master SYBR Green (Cat No. 45323402, Odense, Denmark) were ordered to make complementary DNA (cDNA) and to perform real-time polymerase chain reaction (PCR) respectively. Materials, including mTOR, p-mTOR, 4EBP1, p-4EBP1, ERK1/2, p-ERK1/2, and β-actin antibody, were bought from Santa Cruz (Dallas, TX) for the research.

Pregnancy Inducing:

Prior to mating, all mice were in the estrous cycle(31). Female mice (estrus stage) were matched with adult males in a separate cage during the night to mate. The next morning, despite having a vaginal plug in the female mice, this day was considered the first day of gestation.

Study Design:

After complete assurance of pregnancy, the mice were classified by chance into four groups (15 mice per group): There were 15 mice in each group: Vehicle Receiver Group (Normal saline, DMSO, PEG, TWEEN) intraperitoneally (I.P.), fludrocortisone receiver Group (FCA), mTOR inhibitor receiver Group (30 mg/kg PP242), mTOR inhibitor + fludrocortisone receiver Group (FCA+PP242). Then, male mice were isolated from female mice and pregnancy day is recorded every day for each mouse. Days 4 and 5 of gestation in the early morning after 8:00 A.M. the control group received (Normal saline, DMSO, PEG, TWEEN) whereas (1.5 mg/kg) fludrocortisones(32) was combined in with saline solution and fludrocortisone groups receive this solution intraperitoneally. On the other hand, PP242 (30mg/kg)(33) was intraperitoneally injected in the mice at 8:00 a.m. in the mTOR inhibitor group (PP242) on the 4th and 5th day of gestation and finally, fludrocortisone and PP242 were prescribed in the same state injections and compounds in the FCA+PP242 group. On the Day 5th of gestation, mice were killed by cervical displacement under anesthesia with isoflurane material. Uterine horn specimens were isolated under sterile conditions in mice, and endometrial tissue which included epithelium and stroma cells were mechanically shaved and poured in

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Trizol Ls reagent. Then the samples were placed at −80°C for molecular tests.

RNA isolation and quantitative RT-PCR:

Total RNA was isolated from each sample by TRIzol reagent (invitrogen) and reverse transcription reaction was done with a RT Kit (Thermo Fisher Scientific) Based on the instructions of the manufacturer of the kit. The RT productions (cDNA) were proliferated by real-time quantitative PCR with SYBR green Master Mix (Ampliqon). The primers sequences are displayed in Table 1. To perform sample analysis, the threshold designated according to the exponential stage of productions, and to analyze the data, the $2^{-\Delta\Delta Ct}$ procedure was performed as explained previously. The expression level of mRNAs was normalized to GAPDH mRNA. All reactions were run in triplicate and all tests were done three individually.

Table 1: mRNA Primer sequences (5'-3') used in quantitative real-time PCR

Primer name		Primer sequence (5 to 3)
Msx-1	Forward	TCTCTTAAACCCCTTGCTACACAC
Msx-1	Reverse	GGCCTCTGCACCCTTAGTTT
Hb-Egf[1]	Forward	CCTCTTGCAAATGCCTCCCT
Hb-Egf	Reverse	CCTCCTCTCCTGTGGTACCTAAA
[2]HAND2	Forward	CGACGTGAAAGAGGAGAAGAGG
HAND2	Reverse	CTGCTCTCCTCTTCTTCACTGC
Gapdh [3]	Forward	AATGTGTCCGTCGTGGATCTGA
Gapdh	Reverse	GATGCCTGCTTCACCACCTTCT

Micro RNA: Reverse transcription and real-time PCR:

First, the whole RNA was separated from the instances of each, then the reverse transcriptase reaction was performed for each sample tested by the Thermo Fisher Scientific enzyme, and in short, 2,000 nanograms of the total RNA was reverse-transcribed by the reverse thermo crypt transmitter thermo fisher kit for instances of each. Using factory instructions, the real-time PCR was done with SYBR Green PCR Master Mix (Ampliqon, Odense, Denmark); with a miRNA-specific forward primer and a universal reverse primer that in Table 2 is displayed. The reaction instructions consist of 95 °C for 5 min, followed by 40 cycles of amplification at 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s. U6 small nuclear RNA was applied as an example of internal control, and eventually, to analyze the data, the $2^{-\Delta\Delta Ct}$ procedure was used.

Table 2: miRNA primer sequences (5'-3') used in quantitative real-time PCR

Primer name		**Primer sequence (5 to 3)**
MiR- 200a	[4]STL	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACATCGT
MiR- 200a	Forward	GGGTAACACTGTCTGGTAACGAT
MiR-145	STL	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAT
MiR-145	Forward	TTGAACCCTCATCCT GTGAGCC
MiR- 451	STL	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAA-CTCAG

[1] Heparin-binding EGF-like growth factor
[2] Heart And Neural Crest Derivatives Expressed 2
[3] Glyceraldehyde-3-phosphate dehydrogenase
[4] Stem Loop

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Primer name		Primer sequence (5 to 3)
MiR- 451	Forward	GGAAGATCTTGACAAGGAGGACAGGAGAG
Universal	Reverse	GTGCAGGGTCCGAGGT
U6	Forward	GCTTCGGCAGCACATATACTAAAAT
U6	Reverse	CGCTTCACGAATTTGCGTGTCAT
U6	STL	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAAATAT

Western Blot Analysis:

TRIzol LS was used to isolate total protein from endometrial tissue. Separated protein was homogenized with 200 µl lysis radioimmunoprecipitation assay buffer comprising protease inhibitor cocktail (Sigma Aldrich), and after homogenizing, we centrifuged them at 12,000g at 4°C for 20 min. The protein concentration was calculated using the Bradford method in the obtained sequence (Bio-Rad, San Francisco, CA). An equal value (100 µg) of the entire protein of each sample were isolated on a sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE; 10%) and transferred to a polyvinylidene difluoride (PVDF) membrane. Non-proprietary connections were closed with 5% without fat milk for 2 hr at Laboratory room temperature. Then, the membranes were impregnated with antibodies monoclonal primary antibodies (1:500; Santa Cruz): ERK1/2 (H-72, sc- 292838), p-ERK1/2 (Thr 177, sc-16981-R), mTOR (sc-1550-R), p-mTOR (Ser 2481; sc-293089), 4E-BP1 (sc-997), p-4E-BP1 (sc-293124), and β-actin (sc-47778) as a loading control (1:1000; Sigma Aldrich) overnight at 4°C. After washing with phosphate buffered saline, the membrane was incubated with horseradish peroxidase-conjugated secondary antirabbit antibodies (1:5000; Santa Cruz) for 1 hr. Bands become visible by a boosted chemiluminescence diagnostic kit (Bio-Rad). The density and thickness of the desired bands were measured by the Image Software.

Statistical Analysis:

GraphPad software (GraphPad Prism 8.4.2.679 Win/Mac) was used to analyze the data and differentiate between experimental groups. To analyze statistical importance, two-way analysis of variance with Tukey's post-hoc test was used. All data were expressed as mean ± standard error of mean, and $p < 0.05$ was counted statistically meaningful in all instances.

Results

The expression of miR-200a, mir-145, mir-451, HAND2, ENaC-α and SGK1:

The analysis of real-time PCR revealed that the expression of ENaC-α (*P<0.01*, Figure 1a), and SGK1 (*P<0.001*, Figure 1b) was remarkably decreased in the fludrocortisone-treated sample as compared with the control sample. HAND-2 mRNA expression after consumption of fludrocortisone was a significant difference compared to the vehicle group (*P<0.01*), as it is displayed in Figure 1c.

In fludrocortisone receiver group, miRNA 200a (p < 0.01; Figure 2a), and miRNA-145(p < 0.05; Figure 2c) expression decreased considerably in compared with the vehicle group. But, fludrocortisone consumer group, miRNA-451 (Figure 2b), expression increased considerably in comparison with the vehicle group (*P < 0.001*). The analysis of the gene expression demonstrated the effect of PP242 on the expression of ENaC-α, SGK1, and HAND2. The results showed that there was a statistically meaningful difference between the PP242-treated sample and the control sample, when the expression of ENaC-α (p<0.05, Figure 1a), SGK1 (*p<0.01*, Figure 1b) and HAND2 (*p<0.01*, Figure 1c) was compared. However, in PP242 group, miRNA-451 expression (Figure 2b), decreased considerably in comparison with the control group, with (p<0.05). Also, in PP242 group, miRNA-145 expression (Figure 2c), miRNA 200a expression (Figure 2a) both increased considerably in comparison with the control group, with (*P<0.001*) for both.

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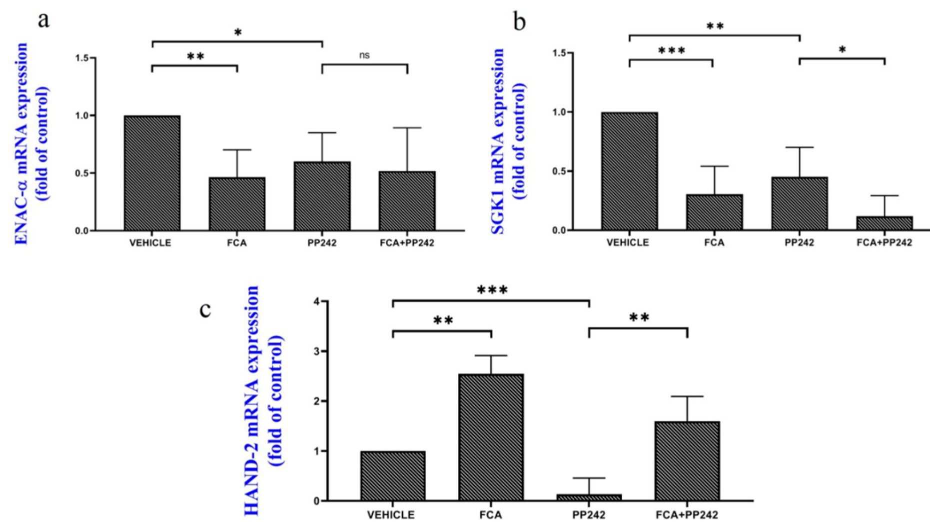

Fig.1. The relative expression of ENaC-α (A), SGK1 (B), and HAND2 (C) in the endometrium of all experimental groups. Data are presented as mean ± SEM (n=3). *p<0.05, **$P<0.001$, ***p<0.0001

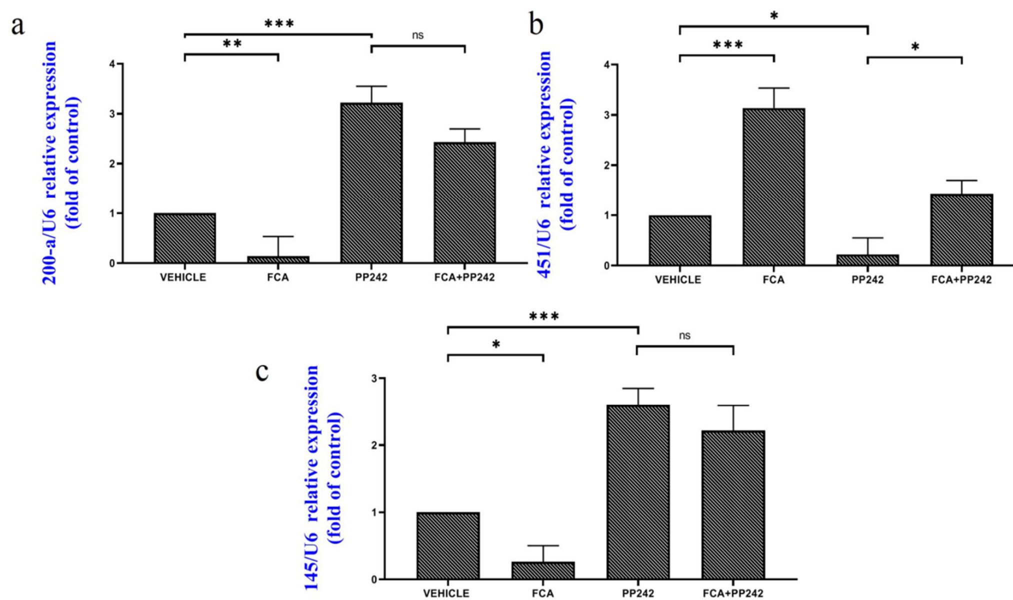

Fig.2. The relative expression of miR-200a (A), miR-451 (B), and miR-145 (C) in the endometrium of all experimental groups. Data are presented as mean ± SEM (n=3). *p<0.05, **$P<0.001$, ***p<0.0001

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[DOI: 10.61186/rabms.10.2.154]

In the FCA+PP242-treated sample, the expression of ENaC-α, SGK1genes did not significantly change in comparison with the PP242-treated sample in Figure 1. HAND-2 mRNA expression after consumption of fludrocortisone was a significant difference compared to the vehicle group (*P<0.01*), as it is displayed in Figure 1c. In FCA PP242 subgroup expression of miRNA-451 ($p < 0.05$; Figure 2b) reduced considerably in comparison with the control group which only received PP242. But miRNA-145 (Figure 2c) and miRNA 200a (Figure 2a) expression did not have any differences compared to the PP242 group.

Western Blot:

In this study, the phosphorylation level of mTOR (p-mTOR) and p-4E-BP1 proteins was also analyzed (Figure 3). The western blot analysis (Figure 3B) demonstrated that the administration of fludrocortisone significantly increased the phosphorylation rate of mTOR compared with the control sample (*p<0.01*). However, the results obtained from the western blot analysis showed that phosphorylated forms of 4E-BP1 protein did not change in the fludrocortisone-treated sample as compared to the control sample (*P=1.00*, Figure 3C).

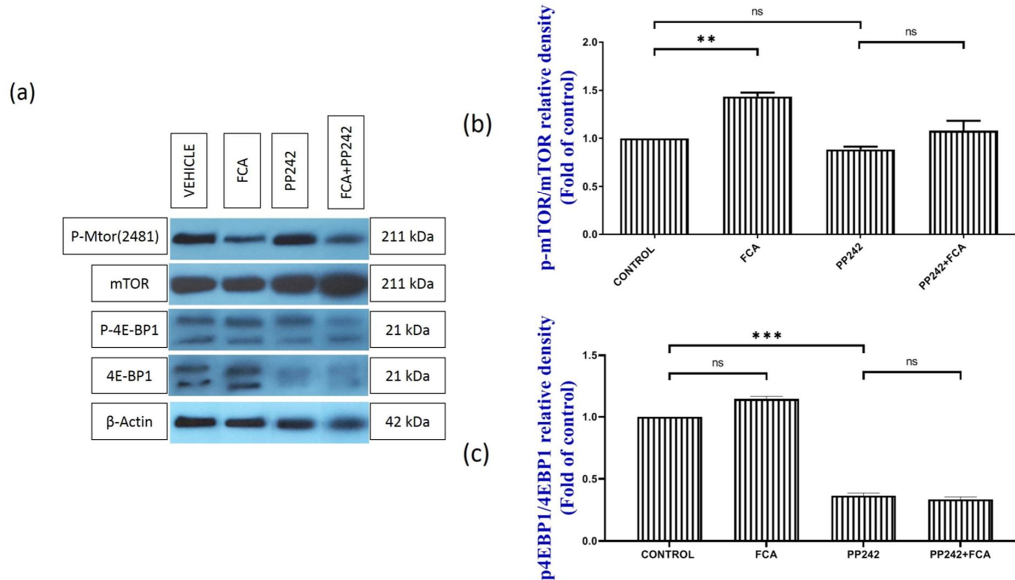

Fig.3. Total protein expression, as well as the phosphorylation level of mTOR and 4E-BP1 in the uterus. (A) Representative images of mTOR, 4E-BP1, and β-Actin proteins. The relative phosphorylation levels of mTOR (B), and 4E-BP1 (C) in the endometrium. Data are presented as means ± SEM (n=3): *$p<0.05$, ***p<0.01*, ****P<0.001*

The western blot analysis (Figure 3B) showed that the administration of PP242 protein did not change the level of mTOR phosphorylation in comparison with the control sample (*P=0.482*). Further analysis revealed that the difference in the level of p-mTOR was not statistically meaningful (*P=0.114*, Figure 3B) between the FCA+PP242-treated and PP242-treated samples. On the other hand, the administration of PP242 significantly decreased the phosphorylation rate of p-4EBP1 in the PP242-treated sample when compared to the control sample (*P<0.001*, Figure 3C). However, the difference in the rate of phosphorylation in p-4EBP1 was not statistically significant between the FCA+PP242-treated and PP242-treated samples (*P=1.00*, Figure 3C).

Discussion

During the early stages of gestation, the endometrial receptivity, implantation, as well as endometrial

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remodeling or decidualization is vital processes for the successful embryonic growth (34, 35). Although the molecular mechanisms underlying the endometrial receptivity remain mostly elusive, it is now known that there are sequences of physiological and morphological alterations, which could ensure the accomplishment of the embryonic implantation (34, 35). A group of these critical genes and factors involved in the implantation process have been previously studied such as ENaCα, SGK1, mTOR, and p-4E-BP1 (29, 36-39).

Glucocorticoids are necessary for normal fertility in the uterus as the ablation of their receptors in the uterus reduces the success rate of implantation (40). Despite the positive impacts of synthetic glucocorticoids on pregnancy outcomes, it may have some negative dose-dependent effects on the implantation process (41, 42). Namdar et al. have reported that high-dose dexamethasone increased the abortion rate during the early stages of pregnancy (43). Moreover, the effects of glucocorticoids and mineralocorticoid on the expression of these genes (SGK1, LIF, and MUC-1) in different tissues have been previously reported (44-47). Therefore, we investigated whether fludrocortisone, which possesses moderate glucocorticoids and potent mineralocorticoid activity, could influence the rate of the endometrial receptivity and the implantation success through modifications in the expression of these genes and proteins. PAS staining is a special staining method in histopathology laboratories for the evaluation of the glycocalyx carbohydrates such as the mucin contents present in the endometrium. Mucins are concentrated at the apical surface of the uterus epithelium, forming a mechanical barrier against microbial insults (48, 49). Due to the anti-adhesive property of MUC1, decreased expression of MUC1 may increase the endometrial receptivity and implantation in the uterus (50). In our previous study, the results confirmed that the administration of fludrocortisone decreased the thickness of the apical glycocalyx in uterine epithelial cells at the implantation process. It was also shown that the administration of PP424 causes an increase in the apical glycocalyx of uterine epithelial(51). The increased thickness of apical glycocalyx prevents the adherence of trophoblast cells to the uterine epithelium during the attachment phases of implantation, as the embryo needs to encounter the epithelial glycocalyx(51, 52). Thus, it can be concluded that fludrocortisone could increase the receptivity of the endometrium during implantation by reducing the amount of mucin and glycocalyx.

Hand2, a transcription factor that is stimulated by the hormone progesterone in uterine stroma, has also been reported to be involved in receptivity, implantation s, and decidualization in mice(19, 53). In addition, in another study, it was shown that on day 3 of pregnancy, the expression of HAND2 occurs strongly in the stroma, but not particularly in the epithelium. Stroma expression continues in implantation and can be detected up to 8.5 days of gestation, indicating the role of HAND2 during decidualization and implantation(19). Other studies have reported that removing Hand2 from the uterus leads to infertility due to implantation defects(18). According to the study, prescribing fludrocortisone increased HAND2 gene expression in the mice uterus in implantation stage.

Various reproductive events and implantation are noticeably influenced by the alteration in the luminal fluid of the uterus (54). A growing body of evidence indicates that estrogen stimulates the secretion of luminal fluid while progesterone incites the absorption of luminal fluid via improvement in expression of ENaC on the surface of glandular epithelial cells and apical surface of lumina (12). Luminal fluid disappears in the pre-implantation time, causing the closure of the lumen and enabling the embryo to create a physical contact with the uterine epithelium before implantation (8). Epithelial ENaC can mediate the uterine fluid absorption, which results in the closure of the uterine lumen and immobilization of blastocysts (10, 11). According to the previous evidence, ENaC is precisely regulated by serum- and glucocorticoid-regulated kinase 1 (SGK1). SGK1 is able to upregulate the expression of ENaC in the endometrial surface epithelium (13, 55). Moreover, SGK1 is involved in the modification of the interaction between blastocyst and endometrial epithelium, resulting in the regulation of the epithelial

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[DOI: 10.61186/rabms.10.2.154]

ion transport, and luminal fluid reabsorption/secretion (56-58). Both clinical and experimental studies have verified that the expression and activity of SGK1 are diminished during the receptivity window (12, 39, 57). The upregulation of the SGK1 gene in the endometrial receptivity period is associated with infertility while the downregulation of this gene is correlated with the embryo implantation (12, 59). It has also been displayed that the expression of SGK1 in infertile women is markedly higher than their fertile counterparts (58).

In the present study, fludrocortisone therapy decreased the mRNA expressions of both ENaC-α and SGK1 genes during implantation in the uterus. Previous studies have shown that in renal epithelial cells SGK1 plays an essential role in the regulation of ENaC-mediated Na transportation by aldosterone (60). Aniko et al. have found that aldosterone causes an up-regulation in the expression of the SGK1 gene in mineralocorticoid target cells (61). Additionally, several studies have indicated that glucocorticoids elevate the expression of the SGK1 gene, as well as ENaC in epithelial cells (62, 63). Frindt and Palmer have also shown that dexamethasone could increase the protein expression of ENaC in cortical collecting duct (64). Thus, fludrocortisone therapy leads to the short-term decrease in the expression of SGK1, as well as the inactivation of ENaC, which could eventually result in implantation.

Our results indicated that PP242 could not induce a noticeable alteration in the mRNA expressions of ENaC-α and SGK1 genes in comparison with the control. Conversely, Gleason et al. presented that the inhibition of mTOR, using PP242 and AZD8055, noticeably decreases the activity of ENaC in the apical membrane of cortical tubule cells (33). Studies indicated that mTOR mediates the activation of ENaC, as well as the phosphorylation of SGK1 protein, while the administration of PP242 activates ENaC-dependent Na+ transport, and reduces the phosphorylation of SGK1 in renal epithelial cells (65). Furthermore, the administration of PP242 in mice knockout for SGK1 fails to inactivate ENaC, suggesting that the mTORC2/SGK1 signaling pathway is indispensable for the regulation of ENaC (13). Therefore, it is likely that fludrocortisone might be involved in this process through the modulation of critical molecules in the mTORC2/SGK1 signaling pathway. For example, the activation of ENAC protein is one of the exemplary methods for the successful implantation in the endometrium.

Most mammalian miRNAs take part in the regulation of gene expression and are involved in numerous cellular processes such as cell growth, differentiation, apoptosis, metabolism, and spatiotemporal tissue structure regulation (66). Based on our results, miR-200a was downregulated after the administration of fludrocortisone. A study by Jimenez at al. highlighted that the miR-200 cluster regulated by both estradiol and progesterone is substantially downregulated in endometrial stromal cells of mice before the implantation process (23). In contrast, it was shown that during in vitro decasualization of human endometrial stromal cells, miR-200a was upregulated (23). In a study performed by Shen et al., they exhibited that the expression of mmu-miR-200a was decreased during days 4 to 6. Such a decrease in the miR-200a expression might have a negative impact on epithelial cells during the embryo implantation (67). The results of our study indicated that the expression of the miR-200a cluster was upregulated when fludrocortisone was co-administrated with PP242, and led to the upregulation of miR-200a. An increase in the expression of miR-200a, regarding the results of other studies, leads to the reduced endometrial receptivity. In addition, our findings showed that the miR-200a expression was decreased when fludrocortisone was applied alone. The downregulation of miR-200a is a prerequisite for the successful implantation while it increases the rate of endometrial receptivity. The online scanning of miRNAs targets

(http://www.targetscan.org/cgibin/targetscan/vert_72/targetscan.cgi?species=Human&mir_vnc=miR-200ab-5p) showed that the potential targets of miR-200a targets are LIFR, SGK1, and SGK2. Thus, fludrocortisone increases the endometrial receptivity, leading to the downregulation of the miRNA-200a,

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[DOI: 10.61186/rabms.10.2.154]

probably via the decrease in the synthesis of important molecules involved in this process such as SGK1. However, more investigations are required to assess the precise mechanism(s) by which the administration fludrocortisone affects the expression of the miRNA-200a and other potential targets in the endometrium.

Li, R and his colleagues showed that, an extensive genome analysis showed significant reduction in miRNA-451 expression in female sex who have had a IVF with decreased endometrial receptivity because of rising concentrations of progesterone on the day of human chorionic gonadotrophin(HCG) Compared to female sex with normal progesterone levels(68). Oestradiol dependent up-regulation of miR-451 was also seen in mouse uteri(69). Another study also showed that miR-451 was obviously up-regulated at the time of implantation window, and by targeting mRNA Ankrd46, it plays an important role in embryo implantation(70). So according to past results, it can be concluded that fludrocortisone unregulated miRNA-451 and leads to increased implantation.

In studies of microRNAs, Revel et al., 2011 showed that miR-145, were approved as high expression, while their anticipated targets, a group of adhesive molecules involved in embryo implantation, were low expression in a group of patients with implantation defects and uterine acceptance(71). In addition, this study showed a threefold increase in the rate of miR-145 expression in Recurrent Implantation Failure patients with women with normal fertility(71). Other studies have also shown that miR-145 affects the attachment and adhesion of the embryo(25) by reducing the number of IGF1 receptors in the endometrium(24). Therefore, according to this data and information, it can be concluded that fludrocortisone will increase uterine acceptability by reducing the expression of miR-145.

Several signaling pathways are implicated in the regulation of genes and proteins expression involved in implantation. The impact of dexamethasone and PP242 on ERK/mTOR/4E-BP1 signaling pathways was also examined in this study (72, 73). In this examination, we analyzed the effect of fludrocortisone and PP242 on the current signaling pathway. The immunoblotting results have shown that fludrocortisone treatment led to a significant up-regulation in the levels of p-mTOR in the uterus during implantation, whereas p-4E-BP1 levels did not considerably change even after the treatment with fludrocortisone. Of note, PP242 reduced the p-4E-BP1 protein in the PP424-treated group as compared to the control group, while no significant discrepancy was found between these two groups concerning the levels of p-mTOR. Regardless of the vital roles of the mTOR signaling pathways in the regulation of proliferation and cell growth, mTOR has also been reported to be associated with the embryo implantation (29). It has been demonstrated that mTOR-deficient embryos die immediately after implantation (29).

In contrast to our results, some studies showed that glucocorticoids, such as dexamethasone, could inhibit the mTOR signaling pathways in hypothalamic organotypic cultures as validated by decreased phosphorylation of 4E-BP1, as a downstream mediator of the mTOR protein, during implantation (74-76). In opposite to these results, another study reported that 4E-BP1 would not be altered by the treatment with dexamethasone (77). Taken together, in line with previous evidence, showing that intrauterine glucocorticoid signaling is essential for maintaining normal uterine function and fertility, our results showed that fludrocortisone did not alter the condition of endometrial receptivity, while fludrocortisone increased the receptivity markers through mTOR signaling pathway during the implantation window.

Conclusion

In this study, it was revealed that the mTOR signaling pathway could contribute to the slightly downregulation of SGK1, ENaC-α, mir451and miR-200a, following the administration of fludrocortisone during the implantation window while it slightly upregulated the expression of miR-451 and HAND2. We also concluded that the mTOR signaling pathway likely plays an essential role in the endometrial receptivity. Upon the inhibition of this pathway, a group of genes responsible for the detachment of the embryo such as miR-200a and mir-145 would be increased, and

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the implantation process would not be successful. In general, the administration of fludrocortisone before the implantation window might improve uterus receptivity, facilitating the process of successful implantation. Further studies are needed to clarify the precise mechanisms underlying the effect of fludrocortisone on implantation.

Funding Source

The authors acknowledge that the financial support of the current research was provided by the immunology Research Center of Tabriz University of Medical Sciences (Grant No: 58538).

Authors' Contributions

M.B. H-S performed the experiments and wrote the manuscript and analyzed the data; N. Sh performed the experiments; and B. N supervised the entire study and applied for the research grant.

Acknowledgments

The present research has been extracted from the Ph.D. thesis of Mohammad Bakhtiar Hesam-Shariati. The current study was conducted in the Immunology Research Center affiliated with the Tabriz University of Medical Sciences.

Conflict of interest

The authors have no conflict of interest in this study.

References

1. Shokrzadeh N. Semi-quantitative analysis of endometrial receptivity marker mRNA expression in the mid-secretory endometrium of patients with uterine fibromas. Afr J Biotechnol 2012;11(23). http://dx.doi.org/10.5897/ajb11.4072
2. Zhang S, Kong S, Lu J, Wang Q, Chen Y, Wang W, et al. Deciphering the molecular basis of uterine receptivity. Mol Reprod Dev 2013;80(1):8–21. http://dx.doi.org/10.1002/mrd.22118
3. Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. N Engl J Med 2001;345(19):1400–8. http://dx.doi.org/10.1056/NEJMra000763
4. Shokrzadeh N, Saidijam M, Dehghan A, Esna-Ashari F, Sanoee MF, Bahmanzadeh M, et al. Semi-quantitative analysis of endometrial HOXA10 and BTEB1 mRNA expressions in the implantation window of patients with endometriosis and myoma. Tehran Univ Med J 2012;69(10):605–12.
5. Wilcox AJ, Weinberg CR, O'Connor JF, Baird DD, Schlatterer JP, Canfield RE, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319(4):189–94. http://dx.doi.org/10.1056/NEJM198807283190401
6. Niknafs B, Afshari F, Dezfulian AR. Morphological and morphometrical changes of endometrim after application of estrogen and progesterone during luteal phase in the superovulated mice. Iran J Reprod Med 2008;6(3):133–42.
7. Toth B, Würfel W, Germeyer A, Hirv K, Makrigiannakis A, Strowitzki T, et al. Ion channels in the endometrium: regulation of endometrial receptivity and embryo implantation. J Reprod Immunol 2011;90(1):517–29.
8. Ruan YC, Chen H, Chan HC. Ion channels in the endometrium: regulation of endometrial receptivity and embryo implantation. Hum Reprod Update 2014;20(4):517–29. http://dx.doi.org/10.1093/humupd/dmu006
9. Ruan YC, Guo JH, Liu X, Zhang R, Tsang LL, Dong D. Activation of the epithelial Na+ channel triggers prostaglandin E 2 release and production required for embryo implantation. Nat Med 2012;18(7).
10. Salleh N, Baines DL, Naftalin RJ, Milligan SR. The hormonal control of uterine luminal fluid secretion and absorption. J Membr Biol 2005;206(1):17–28. http://dx.doi.org/10.1007/s00232-005-0770-7
11. Naftalin RJ, Thiagarajah JR, Pedley KC, Pocock VJ, Milligan SR. Progesterone stimulation of fluid absorption by the rat uterine gland. J Reprod Fertil 2002;123(5):633–8. http://dx.doi.org/10.1530/rep.0.1230633
12. Salker MS, Steel JH, Hosseinzadeh Z, Nautiyal J, Webster Z, Singh Y, et al. Activation of SGK1 in endometrial epithelial cells in response to PI3K/AKT inhibition impairs embryo implantation. Cell Physiol

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[DOI: 10.61186/rabms.10.2.154]

Biochem 2016;39(5):2077–87. http://dx.doi.org/10.1159/000447903

13. Lang F, Pearce D. Regulation of the epithelial Na+ channel by the mTORC2/SGK1 pathway. Nephrol Dial Transplant 2016;31(2):200–5. http://dx.doi.org/10.1093/ndt/gfv270
14. Arroyo JP, Lagnaz D, Ronzaud C, Vázquez N, Ko BS, Moddes L. Nedd4-2 modulates renal Na+-Cl− cotransporter via the aldosterone-SGK1-Nedd4-2 pathway. J Am Soc Nephrol 2011;
15. Gentilini D, Busacca M, Francesco D, Vignali S, Vigano M, Blasio D, et al. Akt and ERK1/2 signaling pathways are involved in endometrial cell migration induced by 17β-estradiol and growth factors. Mol Hum Reprod 2007;13(5):317–22.
16. Wang CY, Tsai AC, Peng CY, Chang YL, Lee KH, Teng CM, et al. Dehydrocostuslactone suppresses angiogenesis in vitro and in vivo through inhibition of Akt/GSK-3β and mTOR signaling pathways. PLoS One 2012;7(2):e31195. http://dx.doi.org/10.1371/journal.pone.0031195
17. Vemulapalli S, Mita A, Alvarado Y, Sankhala K, Mita M. The emerging role of mammalian target of rapamycin inhibitors in the treatment of sarcomas. Target Oncol 2011;6(1):29–39. http://dx.doi.org/10.1007/s11523-011-0179-4
18. Li Q, Kannan A, DeMayo FJ, Lydon JP, Cooke PS, Yamagishi H, et al. The antiproliferative action of progesterone in uterine epithelium is mediated by Hand2. Science 2011;331(6019):912–6. http://dx.doi.org/10.1126/science.1197454
19. Huyen D, Bany B. Evidence for a Conserved Function of Heart-and Neural Crest Derivatives-Expressed Transcript 2 (Hand2) in Mouse and Human Decidualization. Reproduction. 2011;142(2).
20. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. Nat Med 2012;18(12):1754–67. http://dx.doi.org/10.1038/nm.3012
21. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics: MicroRNAs AS BIOMARKERS FOR DIAGNOSTICS. J Cell Physiol 2016;231(1):25–30. http://dx.doi.org/10.1002/jcp.25056
22. Pichler M, Ress AL, Winter E, Stiegelbauer V, Karbiener M, Schwarzenbacher D, et al. MiR-200a regulates epithelial to mesenchymal transition-related gene expression and determines prognosis in colorectal cancer patients. Br J Cancer 2014;110(6):1614–21. http://dx.doi.org/10.1038/bjc.2014.51
23. Jimenez PT, Mainigi MA, Word RA, Kraus WL, Mendelson CR. MiR-200 regulates endometrial development during early pregnancy. Mol Endocrinol 2016;30(9):977–87. http://dx.doi.org/10.1210/me.2016-1050
24. Kang YJ, Lees M, Matthews LC, Kimber SJ, Forbes K, Aplin JD. MiR-145 suppresses embryo-epithelial juxtacrine communication at implantation by modulating maternal IGF1R. J Cell Sci 2015;128(4):804–14. http://dx.doi.org/10.1242/jcs.164004
25. Hull ML, Nisenblat V. Tissue and circulating microRNA influence reproductive function in endometrial disease. Reprod Biomed Online 2013;27(5):515–29. http://dx.doi.org/10.1016/j.rbmo.2013.07.012
26. Hoang B, Benavides A, Shi Y, Yang Y, Frost P, Gera J, et al. The PP242 mammalian target of rapamycin (mTOR) inhibitor activates extracellular signal-regulated kinase (ERK) in multiple myeloma cells via a target of rapamycin complex 1 (TORC1)/eukaryotic translation initiation factor 4E (eIF-4E)/RAF pathway and activation is a mechanism of resistance. J Biol Chem 2012;287(26):21796–805. http://dx.doi.org/10.1074/jbc.M111.304626
27. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 2004;23(18):3151–71. http://dx.doi.org/10.1038/sj.onc.1207542
28. Shokrzadeh N, Alivand MR, Abedelahi A, Shariati H, Niknafs MB. Upregulation of HB-EGF, Msx. 1, and miRNA Let-7a by administration of calcitonin through mTOR and ERK1/2 pathways during a window of implantation in mice. Mol Reprod Dev 2018;85:790–801.
29. Chen X, He J, Ding Y, Zeng L, Gao R, Cheng S, et al. The role of MTOR in mouse uterus during embryo implantation. J Reprod Fertil 2009;138(2):351–6. http://dx.doi.org/10.1530/REP-09-0090

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[DOI: 10.61186/rabms.10.2.154]

30. Kim DM, Chung JH, Yoon SH, Kim HL. Effect of fludrocortisone acetate on reducing serum potassium levels in patients with end-stage renal disease undergoing haemodialysis. Nephrol Dial Transplant 2007;22(11):3273–6. http://dx.doi.org/10.1093/ndt/gfm386
31. Whitten WK. The effect of removal of the olfactory bulbs on the gonads of mice. J Endocrinol 1956;14(2):160–3. http://dx.doi.org/10.1677/joe.0.0140160
32. Thomas CP, Liu KZ, Vats HS. Medroxyprogesterone acetate binds the glucocorticoid receptor to stimulate α-ENaC and sgk1 expression in renal collecting duct epithelia. Am J Physiol-Renal Physiol 2006;290(2):F306–12.
33. Gleason CE, Frindt G, Cheng CJ, Ng M, Kidwai A, Rashmi P, et al. mTORC2 regulates renal tubule sodium uptake by promoting ENaC activity. J Clin Invest 2015;125(1):117–28. http://dx.doi.org/10.1172/JCI73935
34. Singh M, Chaudhry P, Asselin E. Bridging endometrial receptivity and implantation: network of hormones, cytokines, and growth factors. J Endocrinol 2011;210(1):5–14. http://dx.doi.org/10.1530/JOE-10-0461
35. Bazer FW. Uterine receptivity to implantation of blastocysts in mammals. Front Biosci (Schol Ed) 2011;S3(2):745–67. http://dx.doi.org/10.2741/s184
36. Yang ZM, Le SP, Chen DB, Cota J, Siero V, Yasukawa K, et al. Leukemia inhibitory factor, LIF receptor, and gp130 in the mouse uterus during early pregnancy. Mol Reprod Dev 1995;42(4):407–14. http://dx.doi.org/10.1002/mrd.1080420406
37. Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Köntgen F, et al. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359(6390):76–9. http://dx.doi.org/10.1038/359076a0
38. Bastu E, Mutlu MF, Yasa C, Dural O, Nehir Aytan A, Celik C, et al. Role of Mucin 1 and Glycodelin A in recurrent implantation failure. Fertil Steril 2015;103(4):1059-1064.e2. http://dx.doi.org/10.1016/j.fertnstert.2015.01.025
39. Campbell EA, O'Hara L, Catalano RD, Sharkey AM, Freeman TC, Johnson MH. Temporal expression profiling of the uterine luminal epithelium of the pseudo-pregnant mouse suggests receptivity to the fertilized egg is associated with complex transcriptional changes. Hum Reprod 2006;21(10):2495–513. http://dx.doi.org/10.1093/humrep/del195
40. Whirledge SD, Oakley RH, Myers PH, Lydon JP, DeMayo F, Cidlowski JA. Uterine glucocorticoid receptors are critical for fertility in mice through control of embryo implantation and decidualization. Proc Natl Acad Sci U S A 2015;112(49):15166–71. http://dx.doi.org/10.1073/pnas.1508056112
41. Gur C, Diav-Citrin O, Shechtman S, Arnon J, Ornoy A. Pregnancy outcome after first trimester exposure to corticosteroids: a prospective controlled study. Reprod Toxicol 2004;18(1):93–101. http://dx.doi.org/10.1016/j.reprotox.2003.10.007
42. Wichtel JJ, Evans LE, Clark TL. Termination of midterm pregnancy in mares using intra-allantoic dexamethasone. Theriogenology 1988;29(6):1261–7. http://dx.doi.org/10.1016/0093-691x(88)90006-4
43. Ahmadabad HN, Jafari SK, Firizi MN, Abbaspour AR, Gharib FG, Ghobadi Y, et al. Pregnancy outcomes following the administration of high doses of dexamethasone in early pregnancy. Clin Experim Reprod Med 2016;43(1).
44. Itani OA, Liu KZ, Cornish KL, Campbell JR, Thomas CP. Glucocorticoids stimulate human sgk1 gene expression by activation of a GRE in its 5'-flanking region. Am J Physiol Endocrinol Metab 2002;283(5):E971-9. http://dx.doi.org/10.1152/ajpendo.00021.2002
45. Liu F, Aubin JE, Malaval L. Expression of leukemia inhibitory factor (LIF)/interleukin-6 family cytokines and receptors during in vitro osteogenesis: differential regulation by dexamethasone and LIF. Bone 2002;31(1):212–9. http://dx.doi.org/10.1016/s8756-3282(02)00806-2
46. Treon SP, Mollick JA, Urashima M, Teoh G, Chauhan D, Ogata A, et al. Muc-1 core protein is expressed on multiple myeloma cells and is induced by dexamethasone. Blood 1999;93(4):1287–98. http://dx.doi.org/10.1182/blood.v93.4.1287
47. Bamberger AM, Schulte HM, Wullbrand A, Jung R, Beil FU, Bamberger CM. Expression of leukemia

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[DOI: 10.61186/rabms.10.2.154]

inhibitory factor (LIF) and LIF receptor (LIF-R) in the human adrenal cortex: implications for steroidogenesis. Mol Cell Endocrinol 2000;162(1–2):145–9. http://dx.doi.org/10.1016/s0303-7207(00)00200-8

48. Hey NA, Li TC, Devine PL, Graham RA, Saravelos H, Aplin JD. MUC1 in secretory phase endometrium: expression in precisely dated biopsies and flushings from normal and recurrent miscarriage patients. Hum Reprod 1995;10(10):2655–62. http://dx.doi.org/10.1093/oxfordjournals.humrep.a135762
49. Aplin JD, Hey NA, Graham RA. Human endometrial MUC1 carries keratan sulfate: characteristic glycoforms in the luminal epithelium at receptivity. Glycobiology 1998;8(3):269–76. http://dx.doi.org/10.1093/glycob/8.3.269
50. Brayman M, Thathiah A, Carson DD. MUC1: a multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endocrinol 2004;2(1):4. http://dx.doi.org/10.1186/1477-7827-2-4
51. Shariati H, Seghinsara MB, Shokrzadeh AM, Niknafs N. The effect of fludrocortisone on the uterine receptivity partially mediated by ERK1/2-mTOR pathway. J Cellular Physiol 2019;234(11):20098–110.
52. Shariati MBH, Niknafs B, Seghinsara AM, Shokrzadeh N, Alivand MR. Administration of dexamethasone disrupts endometrial receptivity by alteration of expression of miRNA 223, 200a, LIF, Muc1, SGK1, and ENaC via the ERK1/2-mTOR pathway. J Cell Physiol 2019;234(11):19629–39. http://dx.doi.org/10.1002/jcp.28562
53. Lee K, Jeong J, Kwak I, Yu CT, Lanske B, Soegiarto DW, et al. Indian hedgehog is a major mediator of progesterone signaling in the mouse uterus. Nat Genet 2006;38(10):1204–9. http://dx.doi.org/10.1038/ng1874
54. Long JA, Evans HM. The oestrous cycle in the rat and its associated phenomena. University of California Press; 1922.
55. Lang F, Stournaras C. Serum and glucocorticoid inducible kinase, metabolic syndrome, inflammation, and tumor growth. Hormones (Athens) 2013;12(2):160–71. http://dx.doi.org/10.14310/horm.2002.1401
56. Fisher SJ, Giudice LC. SGK1: a fine balancing act for human pregnancy. Nat Med 2011;17(11):1348–9. http://dx.doi.org/10.1038/nm.2549
57. Lou Y, Hu M, Mao L, Zheng Y, Jin F. Involvement of serum glucocorticoid-regulated kinase 1 in reproductive success. FASEB J 2017;31(2):447–56. http://dx.doi.org/10.1096/fj.201600760R
58. Feroze-Zaidi F, Fusi L, Takano M, Higham J, Salker MS, Goto T. Role and regulation of the serum-and glucocorticoid-regulated kinase 1 in fertile and infertile human endometrium. Endocrinology 2007;148(10):5020–9.
59. Salker MS, Christian M, Steel JH, Nautiyal J, Lavery S, Trew G. Deregulation of the serum-and glucocorticoid-inducible kinase SGK1 in the endometrium causes reproductive failure. Nat Med 2011;17(11).
60. Pearce D, Verrey F, Chen SY, Mastroberardino L, Meijer OC, Wang J, et al. Role of SGK in mineralocorticoid-regulated sodium transport. Kidney Int 2000;57(4):1283–9. http://dx.doi.org/10.1046/j.1523-1755.2000.00963.x
61. Náray-Fejes-Tóth A, Fejes-Tóth G. The sgk, an aldosterone-induced gene in mineralocorticoid target cells, regulates the epithelial sodium channel. Kidney Int 2000;57(4):1290–4. http://dx.doi.org/10.1046/j.1523-1755.2000.00964.x
62. Kim SH, Kim KX, Raveendran NN, Wu T, Pondugula SR, Marcus DC. Regulation of ENaC-mediated sodium transport by glucocorticoids in Reissner's membrane epithelium. Am J Physiol Cell Physiol 2009;296(3):C544-57. http://dx.doi.org/10.1152/ajpcell.00338.2008
63. Hinds LR, Chun LE, Woodruff ER, Christensen JA, Hartsock MJ, Spencer RL. Dynamic glucocorticoid-dependent regulation of Sgk1 expression in oligodendrocytes of adult male rat brain by acute stress and time of day. PLoS One 2017;12(4):e0175075. http://dx.doi.org/10.1371/journal.pone.0175075
64. Frindt G, Palmer LG. Regulation of epithelial Na+ channels by adrenal steroids: mineralocorticoid and glucocorticoid effects. Am J Physiol Renal Physiol 2012;302(1):F20-6. http://dx.doi.org/10.1152/ajprenal.00480.2011

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[DOI: 10.61186/rabms.10.2.154]

65. Lu M, Wang J, Jones KT, Ives HE, Feldman ME, Yao LJ, et al. mTOR complex-2 activates ENaC by phosphorylating SGK1. J Am Soc Nephrol 2010;21(5):811–8. http://dx.doi.org/10.1681/ASN.2009111168
66. Friedman RC, Farh KKH, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009;19(1):92–105. http://dx.doi.org/10.1101/gr.082701.108
67. Shen LJ, He JL, Yang DH, Ding YB, Chen XM, Geng YQ, et al. Mmu-microRNA-200a overexpression leads to implantation defect by targeting phosphatase and tensin homolog in mouse uterus. Reprod Sci 2013;20(12):1518–28. http://dx.doi.org/10.1177/1933719113488453
68. Li R, Qiao J, Wang L, Li L, Zhen X, Liu P, et al. MicroRNA array and microarray evaluation of endometrial receptivity in patients with high serum progesterone levels on the day of hCG administration. Reprod Biol Endocrinol 2011;9(1):29. http://dx.doi.org/10.1186/1477-7827-9-29
69. Nothnick WB, Healy C, Hong X. Steroidal regulation of uterine miRNAs is associated with modulation of the miRNA biogenesis components Exportin-5 and Dicer1. Endocrine 2010;37(2):265–73. http://dx.doi.org/10.1007/s12020-009-9293-9
70. Li Z, Jia J, Gou J, Zhao X, Yi T. MicroRNA-451 plays a role in murine embryo implantation through targeting Ankrd46, as implicated by a microarray-based analysis. Fertil Steril 2015;103(3):834-4.e4. http://dx.doi.org/10.1016/j.fertnstert.2014.11.024
71. Revel A, Achache H, Stevens J, Smith Y, Reich R. MicroRNAs are associated with human embryo implantation defects. Hum Reprod 2011;26(10):2830–40. http://dx.doi.org/10.1093/humrep/der255
72. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, Meijer AJ. Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. J Biol Chem 1995;270(5):2320–6. http://dx.doi.org/10.1074/jbc.270.5.2320
73. Mori S, Nada S, Kimura H, Tajima S, Takahashi Y, Kitamura A, et al. The mTOR pathway controls cell proliferation by regulating the FoxO3a transcription factor via SGK1 kinase. PLoS One 2014;9(2):e88891. http://dx.doi.org/10.1371/journal.pone.0088891
74. Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet 2006;7(3):185–99. http://dx.doi.org/10.1038/nrg1808
75. Wang H, Kubica N, Ellisen LW, Jefferson LS, Kimball SR. Dexamethasone represses signaling through the mammalian target of rapamycin in muscle cells by enhancing expression of REDD1. J Biol Chem 2006;281(51):39128–34. http://dx.doi.org/10.1074/jbc.M610023200
76. Shimizu H, Arima H, Ozawa Y, Watanabe M, Banno R, Sugimura Y, et al. Glucocorticoids increase NPY gene expression in the arcuate nucleus by inhibiting mTOR signaling in rat hypothalamic organotypic cultures. Peptides 2010;31(1):145–9. http://dx.doi.org/10.1016/j.peptides.2009.09.036
77. Weichhart T, Haidinger M, Katholnig K, Kopecky C, Poglitsch M, Lassnig C, et al. Inhibition of mTOR blocks the anti-inflammatory effects of glucocorticoids in myeloid immune cells. Blood 2011;117(16):4273–83. http://dx.doi.org/10.1182/blood-2010-09-310888

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