Research Paper

Letrozole and Clomiphene on Endometrial Receptivity during Implantation in Mice

HAIHONG LI, MEI JIN, RUNHUA ZHANG, YAGUANG HU[1,*]

Department of Nursing, Gansu Maternal and Child Health Care Hospital, Lanzhou 730050, Gansu Province, China
[1]Dept Pharm, Gansu Provincial Maternity and Child-care Hospital, Lanzhou 730050, Gansu Province, China

The purpose of this article is to compare the effects of letrozole and clomiphene on the morphology of the endometrium during pre-ovulation and the receptivity of the endometrium during implantation. Discuss the superiority of letrozole over clomiphene for ovulation induction, and provide a theoretical basis for the clinical application of letrozole ovulation induction. The method of this article is to randomly divide 48 mice into 3 groups: letrozole group (n=16), clomiphene group (n=16) and control group (n=16). Pregnancy vibration after ovulation induction d=4.5 h to take the mouse uterus. Scanning electron microscope was used to observe the changes of endometrial pinocytosis and immunohistochemical technique to detect the expression of leukemia inhibitory factor in the endometrium. The results of the study showed that when the number of days of pregnancy in the three groups was the same, both the endometrial pinocytosis and the expression of Interleukin Enhancer Binding Factor in the letrozole group were similar to those in the control group. Among them, the pre-ovulation endometrial thickness in clomiphene group and control group were both 223.43±48.85, 239.66±68.93, while the letrozole group was 190.45±64.61, and the expression of letrozole group was significantly weaker than the previous two groups. Compared with clomiphene ovulation induction, letrozole did not affect the ultrastructure of the mouse endometrium during implantation and the expression of related genes interleukin enhancer binding factor, but the uterine receptivity of the clomiphene group showed a certain inhibitory effect.

Key words: Letrozole reagent, clomiphene reagent, endometrium research, receptivity research

Infertility is mainly caused by endocrine disorders. The clinical symptoms are decreased or non-ovulation, hormonal regulation disorders in the body, and hyperandrogenism, which often lead to a certain amount of blood in the vagina. World Health Organization (WHO) survey data show that the incidence of infertility among married couples worldwide reaches 15 %[1]. Although the test tube technology has gradually developed and matured and has a certain effect on infertility patients, its implantation rate is still maintained at a low level. The European Society of Human Reproduction and Embryology (ESHRE) survey shows that the pregnancy rate of *In Vitro* Fertilization and Embryo Transfer (IVF-ET) is only about 28.5 %. The reason may be that ovarian function declines due to age, poor egg quality and insufficient ability of the endometrium to accept embryos[2,3]. There are many factors that can lead to infertility. Among them, the share caused by ovulation disorders is about 25 %. Clomiphene (CC) and letrozole (LE) are the most widely used clinical ovulation drugs.

At the beginning of the 21st century, scientists discovered for the first time that LE can be used for infertility. Through the difference in experiment on the effect of LE and CC, it was found that LE has a significant therapeutic effect on infertility. Mainly reflected in: accelerated body metabolism, less impact on the permeability of the endometrium[4]. However, the domestic scientific community still has little understanding of the therapeutic effects of the two drugs. LE can cause female patients to produce more eggs in the uterus and increase the pregnancy rate. It is a better therapeutic drug that has appeared in recent years. Compared with CC and anti-estrogens, the effect of LE is very satisfactory. Therefore, LE is considered.

***Address for correspondence**
E-mail: 407568571@qq.com

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It can become a new drug to replace CC, but LE still has certain unknown points that we need to explore, including its healthy growth of female endometrium, stable secretion of endocrine system, and embryonic development[5,6]. In summary, the specific effects of LE in the field of reproduction need to be further verified and summarized.

This article starts with the meaning and characteristics of Triva and CC, explores the difference between the effects of the two on the endometrial receptivity of pregnant mice, speeds up the recovery, and better promotes the medical research problems of female infertility , The main analysis is based on the comparison of the specific experimental results of the case, finds the problems shown by different treatment methods, innovates the means to solve the problems, explores new methods that meet the self-recovery efficacy, and finds the basis of the balance between drug treatment and the side effects on the body. Those who combine organically[7,8], provide valuable technical experience for future female infertility recovery. At the same time, it can also make suggestions for improvements in future medical technology. Through comparative advantage analysis, we can find similarities and differences in research directions, learn advanced experience, and strengthen specialization in this area. It is expected to provide a theoretical basis for the medical field and improve the resistance to postoperative problems.

THEORETICAL BASIS AND METHOD

Core concepts:

Letrozole (LE)

LE is a new generation of highly specific aromatase inhibitors, synthetic benztriazole derivatives. The function is generally achieved by hindering the activity of related enzymes. The measures adopted here are to limit the change of androgens to female hormones, so the percentage of estrogen content in the body will drop a lot[9]. The feedback effect of estrogen content transmitted to the hypothalamus and pituitary gland in the brain will be weakened. The result is an increase in the percentage of the relevant hormone content, which is conducive to the growth of the follicle, thereby promoting the growth of the follicle and the release of the egg at the same time. The increase in androgen content corresponding to the decrease in estrogen changes will increase the sensitivity of the relevant receptors in the ovary to Follicle-stimulating hormone (FSH), and encourage insulin-like growth factor (IGF-1), endocrine and paracrine factors and FSH to work together to provide for follicular growth condition[10].

Ovulation disorders

Ovulation disorders have two meanings: one is the follicle is in the larval stage and does not have the ability to ovulate; the other is that the egg wall of the follicle is thick and cannot be ruptured, and the egg cannot be discharged[11,12]. WHO divides ovulation disorders into three types: Type I ovulation disorder, which is characterized by hypogonadotropic hypogonadism, insufficient secretion of proprioceptive estrogen and the problematic organ usually appears in the hypothalamus or pituitary in the brain; type II is ovulation. Very few or no ovulation at all, the typical symptom is polycystic ovary syndrome (PCOS): Type III is the premature aging of the ovaries due to various factors, manifested by insufficient secretion of high gonadotropins and low estrogen content.

Treatment methods:

Modern medicines understanding of ovulation dysfunction infertility

The treatment of ovulation dysfunction infertility is mainly to induce ovulation, and the main drugs that have been found are LE, CC and some hormone drugs. Among them, CC is a miraculous effect in promoting female ovulation, but this drug makes the ovulation rate high and the embryo survival rate is low. Studies have shown that using CC to treat infertility caused by the inability to produce eggs due to PCOS, the ovulation rate in the CC group increased to 64 %, but the pregnancy rate was only 18 %. Therefore, although CC has a significant effect in promoting ovulation, it will inevitably cause damage to the mother's body. Therefore, it is necessary to find a specific drug with relatively small side effects.

In recent years, Trazole has been used clinically to promote ovulation in anovulatory infertility. Its principle is to act as an aromatase inhibitor to inhibit and reduce the production of estrogen, thereby failing to carry out negative feedback on the hypothalamus-pituitary circuit, leading to the content of endogenous gonadotropin increases suddenly, and the growth and development of follicles is accelerated. But it has the shortcomings of more ovulation and low pregnancy similar to CC. In addition, urogonadotropin (human

menopausal gonadotropin-HMG) and chorionic gonadotropin (HMG) are also clinically used. HMG is extracted from the urine of menopausal women. It is mainly FSH, which can promote follicular development. But for people with PCOS, it is easy to cause complications such as ovarian hyperstimulation syndrome (OHSS).

Human chorionic gonadotropin (HCG) is similar in structure to Luteinizing Hormone (LH), used for those who cannot rupture spontaneously after the follicle matures. However, it is not difficult to find in clinical practice that some patients are resistant to ovulation-stimulating drugs, which cannot solve the problem of immature follicles or non-ovulation after maturity. Therefore, surgical treatment has become the second choice. Common surgical methods are laparoscopic ovarian perforation, ultrasound guided ovulation, and ovarian wedge resection. Because it is an invasive treatment and has certain damage to ovarian function, it is less recommended for use.

Traditional Chinese medicine's (TCM) understanding of ovulation dysfunction infertility

The theory of TCM believes that the kidney stores essence, refines qi, and forms qi. The rise and fall of the essence of the kidney dominate the growth, development and reproduction of the human body. The kidney is the congenital foundation, and the spleen is the acquired foundation, providing the body's essential essence, qi, blood and body fluid. Congenital deficiency or acquired injury decreases fertility. Or usually addicted to eating fat, sweet and thick taste, hurting the spleen and stomach, causing phlegm and dampness to accumulate in the internal organs, loss of qi and blood, and the eggs cannot mature. The heart dominates the mental activities of the human body. The heart's qi and blood are insufficient to promote blood circulation; or feelings are not smooth, angry and sad, the liver qi cannot be adjusted, and the blood is weak, causing blood stasis, phlegm and dampness, and also affecting the follicles growth and development. Insufficient kidney qi or chronic illness, repeated miscarriage, damage to kidney qi, deficiency of kidney essence and qi deficiency, dystrophy of the uterus, inability to inject sperm into pregnancy; or insufficient Kidney Yang, life gate Fire decay hinders the development of the uterus or can't trigger the enthusiasm, or the inadequate kidney qi can lead to insufficiency of the fetus and loss of the fetus. This reflects the idea of endometrial receptivity. At the same time, many doctors have found that kidney-tonifying Chinese medicine can regulate the expression of endometrial cytokines, increase endometrial thickness, regulate endometrial blood flow, and improve endometrial receptivity.

Kidney deficiency is the root cause of ovulation dysfunction infertility. Professor Xia Guicheng believes that ovarian function is closely related to the internal environment of the body. Enrichment of kidney essence is an important condition for follicular development. Raising the level of Kidney Yin and decane water can promote the development of follicles and make them mature. At the same time, they can be adjusted with blood circulation drugs. The function of heart, liver and spleen promotes the transformation of Yin and Yang so that the follicles are discharged smoothly. Numerous physicians have clinically treated ovulatory infertility, and they have observed that the method of invigorating the kidney and promoting blood circulation can increase the diameter of the follicle, and the effect is remarkable.

EXPERIMENTAL VERIFICATION

Experimental materials:

LE, Jiangsu Hengrui Pharmaceutical Co., Ltd; CC; HCG; Normal saline; 10 % formalin solution; Phosphate Buffered Saline (PBS) pH 7.2-7.4, 0.01 M and 0.02 M; 2.5 % glutaraldehyde fixative; Diaminobenzidine (DAB) color developing fluid; 2 % isoamyl acetate; 30 %, 50 %, 70 %, 100 % acetone; Phosphate buffer; Hari hematoxylin and eosin dye

Experimental equipment:

1 ml syringe; Ophthalmic scissors, ophthalmic forceps; Glass slide, 0.5 ml pipette tip; Wet box, slicer; Table top high-speed centrifuge (TCL-16G), Japan; Electric heating constant temperature drying oven GZX-DH, Shanghai Yuejin first medical instrument factory; Optical microscope Olympus, Japan; 4º refrigerator, TCL Group Co., Ltd; Low temperature refrigerator type 938 Forma Scientific; Microwave oven S650 made by Glanz Company; Scanning electron microscope, Olympus, Japan; Critical point dryer Hitachi Japan; Vacuum Coater Japan Hitachi Company.

Experimental process:

Establishment of animal model and acquisition of organization

Select 48 clean mice and randomly divide them into

3 groups with 16 mice in each group. LE group: LE 7 mg/kg d dissolved in 1 ml saline; CC group: CC 140 mg/kg d dissolved in 1 ml saline; control group: 1 ml saline, gavage each for 2 d. Take 6 mice to remove the eyeballs and take blood. After centrifugation, the serum is frozen in a refrigerator at -20º for testing. Then the uterus of each group of mice was taken, fixed with 10 % formalin, and cut into upper, middle, and lower sections to make conventional Hematoxylin and eosin stain (HE) staining slices; the remaining 13 mice in each group were injected with 5 international units (IU) HCG respectively, male and female 1:1 close cage. If a vaginal suppository is found at 7 am the next morning, it will be the first day of pregnancy (D1).

Measurement of endometrial tissue

Take the HE stained sections of the pre-ovulation stage, use color medical graphic analysis to measure and observe the thickness of the mouse endometrium; measure the length, volume and maximum diameter of the glands in the D4.5 HE stained film; observe the characteristics of the endometrium. Immerse the new slide in the cleaning solution for 24 h, rinse it with running clean water 3 times, rinse with distilled water for a long time, soak it in alcohol with an alcohol concentration of 15 % for 3 h, then dry it and place it in a dust free environment for the next time use. Use a rotary microtome to slice the wax block continuously with a thickness of 5 μm and place it on the prepared glass slide. Cut one side of the D4.5 mouse uterus with ophthalmic scissors, put it into the lobules of physiological saline, inhale the physiological saline with a 1 ml syringe, flush the uterus through the cervix, collect the flushing fluid, and observe whether there are embryos. The specific measurement conditions are shown in Table 1 and Table 2.

DATA ANALYSIS

During the experiment, it is necessary to sample the mucosa of the mouse endometrium, but it may inevitably lead to the destruction of the endometrial structure, which caused us a lot of trouble in the staging of the endometrium in the initial stage of the experiment, so the staging method in this experiment only divided the endometrium into three stages: early, middle and late secretion. By comparing the composition ratio of the endometrial staging of mice in different drug groups, it can be obtained: the mice in the CC group have the highest proportion of the membrane in the early secretion period compared with the other two groups. We believe that the reason is the expression of corpus luteum function is induced after taking CC. Obstacles, that is, CC has side effects, namely: it makes the mouse endometrium and follicles grow out of step. The mechanism is mainly due to the existence of an antagonistic effect when CC is effective, which will cause the estrogen not to be fully expressed during the growth of the endometrium, leading to abnormal development cycles, and the embryo and the endometrium in the mother

There is a time difference in follicle growth. To understand the specific implementation mechanism, other experiments are needed, to prove it. After an egg is released for about 6 d, the endometrium is medically called the secretory period, and the secretory period can also be divided into three stages: early, mid, and late

TABLE 1: THE MORPHOLOGICAL RESULTS OF THE ENDOMETRIUM IN THE PRE-OVULATION AND IMPLANTATION STAGES OF EACH GROUP

Group	LE group	CC group	Control group	p
Intimal Thickness (μm)	190.45±64.61	239.66±68.93	223.43±48.85	0.16^{a} 0.03^{b} 0.49^{c}
D4.5 Gland Area (μm^2)	1693.78±383.59	1415.13±273.83	1815.24±370.07	0.35^{a} 0.04^{b} 0.005^{c}
D4.5 Gland Circumference (μm)	173.73±23.37	159.13±25.77	184.69±16.23	0.18^{a} 0.08^{b} 0.005^{c}
D4.5 Gland Diameter (μm)	51.62±17.03	44.32±9.85	55.90±11.75	0.40^{a} 0.12^{b} 0.02^{c}

a: Comparison between LE group and control group, b: Comparison of CC group and LE group, c: Comparison between control group and CC group

TABLE 2: RESULTS OF PRE-OVULATION AND IMPLANTATION SEX HORMONE LEVELS IN EACH GROUP

Group	LE group	CC group	Control group	p
Pre-Ovulation E2 (pg/ml)	197.82±29.70	338.32±17.04	227.31±24.26	0.12^{a}<0.001^{bc}
Pre-Ovulation LH (mIU/ml)	0.142±0.11	0.163±0.13	0.139±0.071	0.96^{a} 0.80^{b} 0.76^{c}
Pre-Ovulation T (ng/dl)	93.75±28.87	51.50±5.07	59.00±8.52	$0.02^{a}$$0.008^{b}$ 0.56^{c}
D4.5 E2 (pg/ml)	172.03±37.77	321.39±72.96	195.99±11.06	0.40^{a}<0.001^{bc}
D4.5 (ng/ml)	30.21±9.72	44.16±17.45	25.07±10.22	0.50^{a} 0.08^{b} 0.02^{c}

a: Comparison between LE group and control group b: Comparison of CC group and LE group, c: Comparison between control group and CC group

according to the morphological structure. Among the 9 endometrial specimens in the LE group, there were 3 cases in the early secretion period, 5 cases in the mid secretion period, and 1 case in the late secretion period. Among the 11 endometrial specimens in the CC group, 7 cases were in the early secretion period, 3 cases were in the mid secretion period, and 1 case was in the late secretion period. Among the 12 endometrial specimens in the control group, there were 5 cases in the dry secretion period, 6 cases in the mid secretion period, and 1 case in the late secretion period. The comparison found that the composition ratios of the three groups were similar as shown in fig. 1.

As integrin Alpha-v beta-3 (αVβ3) is dominant in the endometrium, we can conclude that insufficient secretion of integrin αVβ3 when the endometrium is in a critical period is a relatively common phenomenon in mothers who cannot become pregnant. The percentage of integrin αVβ3 in the endometrium of the mother with ectopic disease is much less than that of normal. The role of integrin αVβ3 in the endometrium in the critical period of infertile mothers with hydrosalpinx inflammatory hydrops is not completed and the effect is not completely restored to normal levels after the inflammation disappears. The differential effect of integrin αVβ3 on the endometrium of patients with PCOS problems can also lead to a decrease in the receptivity of the endometrium in the mother. When the endometrium is at a critical stage, the site of action of integrin αVβ3 is in the cell cytoplasm. The effects of mice in the LE and CC groups were similar to those in the control group. In addition, this experiment observed that integrin αVβ3 was positively expressed in the cytoplasm of 2 cases of glandular epithelial cells and 2 cases of luminal epithelial cells in the LE group, 1 case of glandular epithelial cells in the CC group and 1 case of luminal epithelial cells in the control group. The positive expression rate was 18.75 %, as shown in fig. 2.

The experimental results also show that the mode and intensity of action of integrin αVβ3 at the key stage of the mouse endometrium after taking LE or CC is not particularly obvious with that of the control group's natural menstrual cycle. The difference indicates that LE and CC do not have any adverse reactions to embryo implantation in the endometrium at the critical stage. In other words, LE and CC do not interfere with the attachment of embryos to the endometrium at key stages.

As shown in fig. 3, the expression of Tissue inhibitor of metalloproteinases-3 (TIMP-3) in the endometrium of the mice taking CC was significantly higher than that of the natural menstrual cycle of mice taking LE and the control group, while the mice taking the LE group were similar to those of the control group. It can be concluded that CC can lead to an increase in TIMP-3 expression, which is same as our experimental results. After taking CC, the content of TIMP-3 in the mouse maternal endometrium increases, which has adverse effects on the embryos of young mice. CC prevents the embryo from further attaching to the endometrium by enhancing the effect of TIMP-3 in the key stage of the endometrium, so it will lead to juvenile. The adhesion

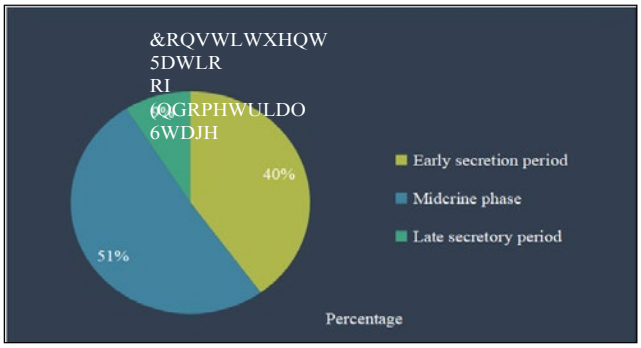

Fig. 1: The composition ratio of endometrial staging

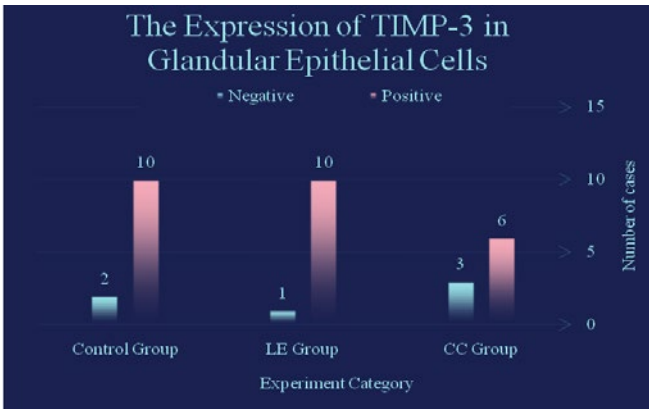

Fig. 2: The expression of integrin αVβ3 in glandular epithelial cells

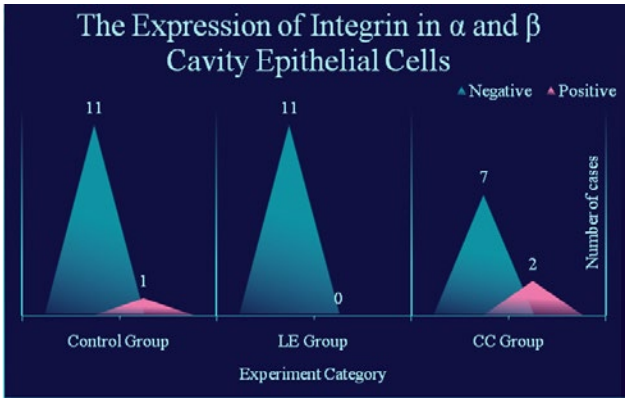

Fig. 3: The expression of integrin αVβ3 in luminal epithelial cells

strength of mouse embryos is not enough; while LE has no obvious effect on the endometrium TIMP-3, the baby mouse peptide fetus can quickly attach to the endometrium to complete the attachment.

As shown in fig. 4, TIMP-3 plays a role in the glandular epithelium, luminal epithelium and mesenchymal cytoplasm of the endometrium at the critical stage. Related experimental verifications have shown that TIMP-3 completely acts on all cells in the endometrium of the entire mouse menstrual cycle. When the endometrium is in the window period, its effect will be significantly reduced, and the effect of related matrix proteases will therefore be enhanced. The decomposition of cell structure is accelerated, which promotes the attachment of the embryo to the deep layer of the endometrium, and facilitates the fixation of the embryo. The role of TIMP-3 in the endometrial tissue of the CC group is superior to that of the LE group and the control group, and the expression of the LE group and the control group is similar.

In short, the experimental results show that the follicular development rate during the treatment of LE ovulation is lower than that of CC, and the pregnancy rate is similar to that of CC; it has little adverse effect on cervical mucus secretion and endometrial growth. As shown in fig. 5, it basically does not interfere with the attachment of embryos to the endometrium at the window stage; although CC does not interfere with the attachment of young mouse embryos to the endometrium, it hinders its deep infiltration into the endometrium. Part of the treatment effect of CC is not good after switching to LE can have satisfactory results. Therefore, LE can be used as a supplement for CC.

RESULTS AND DISCUSSION

LE is a new generation of highly specific aromatase inhibitors. The mechanism of action of LE is to promote the discharge of eggs by acting on the central mechanism. In the central system, by inhibiting aromatase activity, inhibiting the conversion of androgens to estrogen, reducing the content of estrogen in mice, and further inhibiting the negative feedback effect on the hypothalamus and or pituitary gland through estrogen increases the promotion. The synthesis and production of gonadal hormones greatly accelerate the development of follicles in the periphery, the androgen content in the ovaries increases rapidly, the sensitivity of the follicles increases, and the expansion of FSH directly leads to the development and maturity of the follicles, thereby promoting the synthesis of estrogen. Secondly, the increase in androgen content will stimulate the secretion of IGF-1, and endocrine and paracrine factors work with FSH to promote follicular development and ovulation, because of the short half-life of LE (45 h), and no antagonism similar to CC. Therefore, it can be considered that the effect of LE is much better than that of CC in terms of promoting maternal ovulation. Mainly manifested in the small impact on the endometrium, high ovulation induction pregnancy rate and reduced risk of multiple pregnancies, so it can add a treatment option for infertility patients. By selecting mice to simulate the LE and CC ovulation cycle, comparing the effects of the two ovulation-stimulating drugs on the endometrial receptivity, it was found that after LE ovulation, although the estradiol (E2) level was lower than that of the CC group, the endometrium was better than the CC group, which is believed to be related to the different mechanisms of action of the two ovulation-stimulating drugs. LE has no anti-estrogen effect similar to CC.

Aiming at the E2 level and the degree of endometrial development, experimental verification found that the

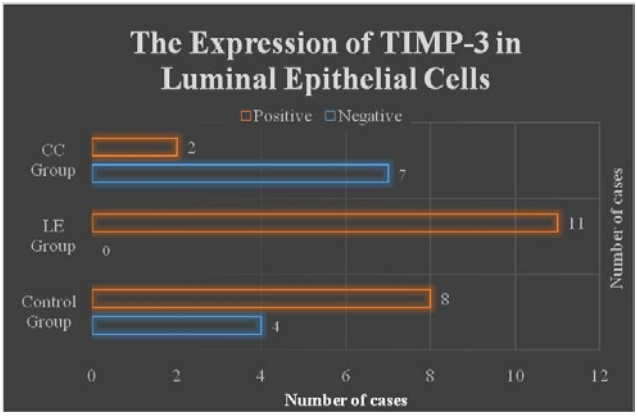

Fig. 4: TIMP-3 expression in luminal epithelial cells

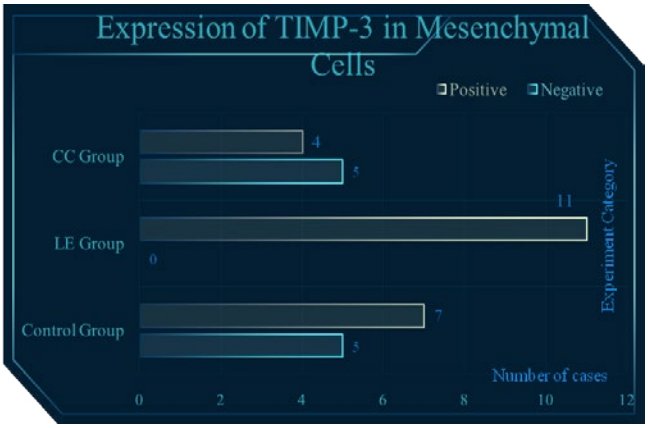

Fig. 5: TIMP-3 expression in mesenchymal cells

combination of mice taking CC in the pre-ovulation period has relatively lower levels than the LE group. The results of this group are not exactly the same as the conclusions reached by some researchers. The reason may be that the selected mice in this group are Kunming mice. Due to ethnic diversity, there are certain differences in sexual cycles. The estrus cycle of the mice used in this group of experiments is only 4-5 d, which corresponds to the maturation time of follicles. It will be shortened accordingly, and the result is that the taken LE is not completely digested and absorbed by the individual, and the gene form is not completely expressed. According to the experimental data, by observing the appearance of the endometrium of mice in the LE group, the mice in the CC group, and the normal control group, we can know that compared with the normal feeding group, the endometrial glands of the mice in the LE group were similar in shape, size, peripheral length and longest diameter. In comparison, the mice in the CC group were significantly different. At the same time, a comparison of developmental maturity showed that the endometrial development of mice in the CC group was slower. From this, it can be seen that the effects of LE mice on the body during implantation during ovulation promotion are small, which is beneficial to the synchronization of the endometrium and embryonic development. This is because the LE half-life is very short and the body metabolizes it. Slowly, this mechanism is closely related to the mutual restriction and mutual balance of the inherent hormones in the body during implantation, and the receptors can also express and grow normally. But this does not mean that just because of the thickness of the endometrium during the pre-ovulation period is different, the effect of the two on ovulation induction is obtained. The endometrium has extremely high requirements for embryo implantation, which is directly proportional to the receptivity of the endometrium, what we call the implantation window. Through recent scientific experiments and the conclusions of researchers, long and mature follicles appear in the endometrium at a certain moment, which can be said to be an ultrastructural sign of the opening of the endometrium implantation window.

By comparison, it can be concluded that when the mice are in the implantation state, the endometrial follicles of the mice in the LE group and the control group are basically at the same period, while the female mice taking CC, the development of endometrial follicles is obviously poor, and lags behind the formation of embryos. It can be concluded that the action mechanism of LE is better for promoting the discharge of eggs. Specifically, when the mouse is in the implantation window, we look at the expression of leukemia inhibitory factor (LIF) in the mouse endometrium at this time and find that it is more active. Under normal circumstances, the initial role of LIF in mammalian endometrium is often consistent with the opening time of the animal embryo implantation window, indicating that the expression of LIF in mouse endometrium is closely related to normal development. In the experiment, by observing the expression of LIF on the endometrium of the three groups of mice, it was found that the LIF expression intensity of endometrial epithelial and glandular epithelial cells in the LE group and the control group was similar; while the expression intensity of LIF in the endometrium of the CC group are significantly weaker than the first two groups. It can be seen that taking LE can promote ovulation while making related genes function normally. That is to say, from a microscopic point of view, LE also has certain advantages in promoting ovulation compared with CC, and it can be inferred that the role of LIF gene whether it is mainly affected by the hormone secreted by the uterine carrier and has nothing to do with the embryo itself. A very important point in the mechanism of CC is that it changes the secretion of sex hormone levels in the embryo's maternal body. Hormone levels in turn change the expression of related genes in the endometrium, causing the development of the endometrium to significantly lag behind the development of the embryo. Estrogen and progesterone act on the endometrium through their respective intracellular receptors to make the endometrial glands and stroma hyperplasia, regulate the development of the endometrium, and provide the material basis for embryo implantation. CC reduces the expression of estrogen receptor (ER) and progesterone receptor (PR) in the endometrial cells at the late secretory stage, causing the endometrium to lack the normal response to sex hormones E2 and progesterone (P), and the endometrial dysplasia affects embryo implantation.

To sum up, although LE and CC affect the endometrial development in the early stage of ovulation, the former has no obvious weakening effect on the mechanism of ER and PR during development, that is to say, Mice taking LE have normal secretion hormone levels, normal endometrial development, normal endometrial molecular structure expression during pregnancy, and normal LIF function. Endometrial development is consistent with the progress of mouse embryos. The receptivity of the membrane is significantly improved compared with clomiphene, and it is suitable for

embryo implantation. In addition, the viewpoints demonstrated in this article are all based on mice. Whether the effects on humans are the same or not requires further experimental verification. However, this article provides certain theoretical and data support for the further widespread application of LE.

Author's contributions:

Haihong Li and Mei Jin, contributed equally to this work.

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None

Conflict of Interests:

The authors declared no conflict of interest.

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