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Review Article

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Leukemia Inhibitory Factor (LIF) Modulation: a Novel, Non-Hormonal Contraceptive Method

Abstract

Background: Numerous studies have demonstrated that Leukemia Inhibitory factor (LIF) plays an essential role in embryonic implantation. Vaginal application of pegylated LIF antagonists can successfully prevent implantation and pregnancy in mice. The development of non-hormonal, female-controlled contraceptives is imperative, as combined oral contraceptives are associated with depression and are not feasible for use in all women.

Methods: This paper reviews 44 studies regarding LIF, implantation, hormonal contraceptives and the use of LIF antagonists as a means to inhibit pregnancy.

Summary: Current research indicates that LIF-modulation could be effective as a non-hormonal contraceptive method, although researchers should be wary of the negative side effects associated with systemic LIF modulation. Vaginal application of LIF antagonists could decrease the risk of negative side effects.

Introduction

Reciprocal molecular communication between an implantation competent blastocyst and a receptive uterus is imperative for proper embryo implantation. Problems during implantation can result in miscarriage or pregnancy associated disorders. (1) Proper implantation depends on both maternally and embryonically produced factors. The best known maternal factors are ovarian estrogen and progesterone, but factors secreted from the uterine glands such as Leukemia Inhibitory Factor (LIF) are also vital for successful implantation. (2,3)

This review aims to provide a brief overview of current knowledge regarding the structure of uterine glands and their role in establishing pregnancy, specifically through their secretion of leukemia inhibitory factor (LIF) during implantation and decidualization[1]. LIF may also act as a trophic factor for the embryo. (6,7) Next, this review aims to discuss whether LIF could be a viable alternative to current forms of hormonal contraception, specifically, combined oral contraceptives. (COC) Various adverse side effects of COC have been identified including depression, which is the most commonly cited reason for discontinuation. (36) Other side effects like thromboembolism and hypertension have been described but these are less common. (8) Moreover, COC usage is contraindicated in women with known familial thrombophilia and other health conditions such as migraines with aura. Therefore, women with these conditions could benefit from the development of alternative contraceptives. (44)

Uterine Structure

Before discussing the uterine glands and LIF, the general structure of the uterus must be outlined. The uterine wall can be divided into two distinct compartments: the inner mucosal lining (the endometrium) and the smooth muscle outer wall (the myometrium). The endometrium comprises two epithelial cell types: the luminal epithelium (LE) and glandular epithelium (GE). (10) The adult human endometrium can also be stratified into two distinct structural zones: the stratum functionalis and the subjacent stratum basalis. The stratum functionalis contains LE, stroma and branched uterine glands and, unlike the stratum basalis, is shed during menstruation. The stratum basalis thus serves as the generative base for adenogenesis, or uterine gland development, which forms tubular glands. During the proliferative phase of the menstrual cycle, these tubular glands undergo extensive branching to form coiled, branched structures termed "glands" within the loosely packed region of the stratum functionalis called the stratum spongiosum. (10,11)

Due to the ethical constraints related to human research, most experimental studies on the role of uterine glands and their secretions are conducted in mice. Unlike humans, mice are born with a simple epithelial uterus that lacks endometrial glands. Invagination of the luminal epithelium at postnatal day 7 results in the formation of tubular uterine glands. (10,11) As mice do not menstruate, they do not rebuild these glands cyclically.

Leukemia Inhibitory Factor

Vital to implantation

The vital role of uterine glands in establishing pregnancy is largely attributed to their expression and secretion of leukemia inhibitory factor (LIF). (2) LIF is a cytokine of the interleukin-6 family and one of the few molecules that are obligatory for fertility in mice. (9) In a seminal study by Stewart *et al.*, homozygous LIF-null mutant female mice were found to be infertile, even when mated with wild type males.(12) Infertility was found to be caused by failure in implantation, rather than in ovulation or fertilization as fertilized, unimplanted blastocysts could be recovered from LIF-null uteri on GE day 4-7. These recovered mutant blastocysts could implant successfully and develop to term when transferred to wild type females, demonstrating that maternal LIF, rather than embryonic LIF, is required for proper implantation.

LIF is also important for implantation in humans. Examination of fluid within the uterine lumen demonstrated that infertile women secrete significantly less LIF than fertile women. (13) Similarly, women undergoing in-vitro fertilization (IVF) with relatively high levels of endometrial LIF during the window of implantation are more likely to become pregnant than their counterparts. (14,15)

Expression and function

Uterine LIF levels peak biphasically. First, LIF is expressed in the murine uterus at ovulation. However, LIF deficiency has not been shown to affect ovulation. Next and seemingly more important, LIF peaks in the GE during the window of receptivity, which immediately precedes implantation and occurs on day 4 of gestation in mice. This second peak occurs in response to the nidatory estrogen pulse[2] and is essential to rendering the luminal epithelium receptive to embryo attachment. In fact, LIF repletion in ovariectomized mice can compensate for missing nidatory estrogen, demonstrating that vital uterine changes triggered by the nidatory estrogen surge in mice are largely mediated through LIF. (16) LIF is also expressed in the decidualizing stroma during the attachment reactions. (17) In humans, LIF is most abundantly expressed in the GE of fertile women during the secretory or postovulatory phase between days 18 and 28 of the menstrual cycle, analogous to the murine "window of implantation" (18,19)

Secreted LIF functions by binding to its cognate transmembrane receptor (LIFR), which is localized on the apical membrane of the LE. The LIFR-LIF complex then binds to glycoprotein 130 (gp130) to form the activated LIF-LIFR-gp130 complex. (5,20) This complex modulates myriad downstream pathways in the endometrium, such as JAK-stat, wnt/B-catenin, notch and mTOR signalling. (5) For instance, upon LIF-LIFR-gp130 binding, STAT proteins are tyrosine-phosphorylated, homodimerized, and translocated to the nucleus, where they modulate the expression of target genes. Deletions of the gp130 region responsible for STAT phosphorylation also results in failures during implantation, demonstrating that this LIF-activated pathway is vital. (21,22)

LIF-effected pathways are implicated in diverse processes allowing for successful implantation. For example, LIF is postulated to modulate adhesion between endometrial epithelial cells and trophoblast cells. Further, LIF plays a vital role in stromal cell decidualization as LIF-/- mice do not undergo decidualization. (5) While it is still not clear which of LIF's many functions are truly essential to implantation, LIF has repeatedly and conclusively been shown to be vital for successful adhesion, decidualization and thus, implantation. (2,3,23)

Leukemia Inhibitory Factor Antagonists as an Alternative to Hormonal Contraceptives

Because of LIF's vital role in implantation, LIF-modulation to prevent implantation is being explored as a novel, non-hormonal contraceptive method. (5,12)

Previously, Fairlie *et al.* used alanine scanning mutagenesis[3] to target residues within the C and D helices of LIF's four helical structure which are responsible for the species specific binding affinity of human LIF (hLIF) to human LIFR.(20) Using this method, key protein regions and amino acids essential for LIF-LIFR and LIF-gp130 binding were identified. Importantly, this study found that abolition of the identified gp130 binding site in hLIF required for productive LIF-signalling, combined with mutations to increase the protein's affinity to LIFR by >1000-fold generated a potent antagonist of LIF action. This protein will henceforth be referred to as LIF antagonist (LA).

LA has been shown to have a half-life of 10 to 30 minutes. Consequently, effective inhibition of LIF activity and, thus, blastocyst implantation in mice using LA requires continuous LA administration. This can be accomplished by using miniosmotic pumps implanted within the peritoneal cavity, in combination with 4 hourly intraperitoneal LA injections. (21) Evidently, continuous LA administration would not be practical. Therefore, the instability of LA could negate its utility as an alternative, non-hormonal contraceptive.

Fortunately, as shown by Reddy *et al.*, covalent attachment of a branched 40-kd polyethylene glycol moiety (PEG) through a process referred to as "PEGylation" can increase the stability of circulating cytokines. (24) Reddy *et al.* showed that PEGylation of an interferon (IFN) used to treat Chronic Hepatitis C (CHC) resulted in sustained IFN absorption, reduced IFN clearance from the plasma, and higher IFN concentrations in blood plasma when compared to non-PEGylated IFN. (24) By increasing molecular size, PEGylation reduces renal ultrafiltration of attached compounds, decreases uptake of the compound by the liver and protects the compound from proteolytic cleavage. (21) In the study described above, the increased stability of PEGylated IFN meant that the cytokine could be administered less frequently and still be effective in treating CHC; PEGylated IFN was effective when administered once weekly, whereas IFN alone was required three times a week. (24)

In order to increase the stability, and thus, potential utility of LA as a contraceptive, White *et al.* conjugated LA with two PEG molecules (henceforth referred to as PEGLA). (21) Strikingly, mated mice who received only 3 intraperitoneal injections of PEGLA and did not receive a transplanted miniosmotic pump had no implantation sites. Thus, while LA administration for contraceptive purposes may not be feasible in humans, PEGLA administration could be both effective and feasible.

Rather than injecting PEGLA, some researchers have attempted to develop a "birth control vaccine". This would allow one's own immune system to target and neutralize endogenous LIF. Notably, Lemon and Naz induced a LIF- or LIFR-directed immune response by conjugating LIF or LIFR peptides to T cell carrier proteins. (6) While this approach significantly decreased fertility, it did not totally abolish implantation. However, this may not be the case in humans because they are less fertile than mice.

While most studies to date have been conducted in mice, LIF antagonism using antibodies has been shown to inhibit implantation in rhesus monkeys. (25) An in-vitro study by Lalitkumar *et al.* demonstrated that PEGLA can inhibit embryo attachment in humans. In this study, three-dimensional cell culture models on endometrial tissue and excess IVF embryos were combined either with or without PEGLA. (7) PEGLA-treated samples saw failure in embryo attachment to culture while control samples did not. Further, PEGLA treated blastocysts failed to hatch, or emerge from the zona pellucida, and expand. Unlike control blastocysts, PEGLA-treated blastocysts lost phospho-AKT-1 which is a vital factor for cell survival. Further, blastocysts exposed to PEGLA underwent apoptosis at significantly higher rates as indicated by increased caspase-3 activity. Taken together, these points allude to the trophic role of LIF on human embryos as well as affirm its effects on human uterine receptivity. (7) This finding supports that inhibition of LIF activity could prevent implantation in humans. However, LIF-modulation for contraceptive purposes could have certain risks. LIF is implicated in a plethora of non-reproductive pathways. Modulation of these pathways via systemic LIF-modulation, either through a vaccine or intraperitoneal PEGLA administration, could result in adverse side effects. (9,12,18,26,27) These risks would likely increase when LIF is modulated over an extended period as would be necessary for contraceptive purposes.

For example, LIF is produced by astrocytes in response to autoimmune insults within the central nervous system and has been shown to increase oligodendroglial survival in-vitro. Similarly, LIF has been shown to prevent oligodendrocyte death in animal models of multiple sclerosis (MS). (28) Systemic administration of LIF antagonists over four days has been shown to worsen experimental autoimmune encephalomyelitis (EAE), which is an experimental model of autoimmune disease induced by immunization against myelin epitopes in mammals. EAE is a standard animal model for multiple sclerosis (28), doubling observed oligodendrocyte loss. (29) Thus, any LIF-targeting contraceptive which would systematically inhibit LIF action could have negative effects on oligodendrocyte survival and thus, myelin integrity.

Moreover, LIFR-null mutant mice have been shown to have a reduced number of spinal and brainstem astrocytes. (9)

Further, LIF may play a key role in modulating hematopoiesis. LIF has been shown to be constitutively expressed in bone marrow stroma. Exogenous LIF administration has been shown to increase platelet counts in a dose-dependent manner. (26) Thus, systemic LIF modulation for contraceptive purposes could potentially alter blood composition. To my knowl-

edge, this effect has yet to be studied in LIF-null mice.

However, LIF exhibits significant homology with Oncostatin M and Ciliary Neurotrophic Factor, both of which are secreted factors that can bind the LIFR. Therefore, the effect of LIF modulation on the non-reproductive systems described above could be dampened by other homologous factors binding to LIFR.(12) In fact, despite the many potential issues of systemic LIF modulation, LIF-null mice in Colin Stewart's seminal LIF-knockout study appeared normal, although slightly smaller than their wild type counterparts. (12) However, in Stewart's study, the non-reproductive health of the mice was not examined closely. (12)

Nevertheless, Menkhorst *et al.* demonstrated that intraperitoneal injections of PEGLA (like those performed by White *et al.*) affect bone remodelling. (9,21) Specifically, intraperitoneal injections of PEGLA resulted in increased cancellous bone volume and thickness. Further, Menkhorst *et al.* observed decreased osteoclast levels in females injected with PEGLA. (9) Non-mated females treated with PEGLA also had less osteoid[4] and osteoblasts than controls. This effect was not observed in mated females, as control-mated females already had low osteoblast and osteoid levels. Taken together, these results indicate that intraperitoneal injection of PEGLA decreased bone remodelling. This is a notable finding with regards to PEGLA's potential use in women as a contraceptive as low levels of bone remodelling increase the risk of fracture in adult humans. (29) More generally, this finding demonstrates that PEGLA and LIF-modulating vaccinations can affect systems outside of the uterus.

Previous research has shown that vaginally administered drugs preferentially localize to the uterus in what is termed as the "uterine first pass effect." (9,30) The preferred explanation for this effect is that drugs administered via the vagina are absorbed through veins within the upper third of the vagina and are transported by countercurrent exchange (exchange by diffusion between two fluids flowing in opposite direction) to the uterine arteries. However, the exact mechanism of the "uterine first pass effect" is still unclear. (9) Possible explanations for this phenomenon include direct diffusion of the compound through tissues, passage of the compound to the uterine lumen through the cervical lumen, and transport of the compound via venous or lymphatic circulatory systems (the lymph system has been recognized as an important carrier for steroid hormones). (30) Regardless of the exact mechanism of the "uterine first pass effect", this phenomenon can be exploited to mitigate the potential systemic effects of PEGLA application. In fact, vaginal application of PEGLA results in its localization to the uterine luminal epithelium and the basal surface of the glandular epithelium as opposed to its localization to the liver, ovary, oviduct, spleen and thyroid, which was the case in IP injected non-mated mice. (9) Strikingly, in Menkhorst *et al.*'s study, mice that received PEGLA vaginally did not have any change in cancellous bone volume or thickness or in osteoclast/blast number and size when compared to controls, while mice receiving PEGLA intraperitoneally did. (9) This demonstrates that systematic effects of LIF modulation by PEGLA can be mitigated by vaginal administration of the compound. Furthermore, vaginal administration of PEGLA still effectively inhibited implantation, demonstrating that PEGLA can be absorbed through the vagina to inhibit LIF signalling and therefore, implantation in the uterus. (9)

If LIF-modulating contraceptives are to be brought to market, future studies on LIF modulation or using LIF-knockout mice, ought to more thoroughly investigate the overall health of the organism rather than only focusing on implantation. For instance, while Menkhorst *et al.* showed that both intraperitoneal and vaginal administration of PEGLA did not exacerbate murine EAE, the effects of long-term systemic PEGLA exposure, or LIF modulation in general, on the CNS are yet to be explored. (9) Given that the effects of LIF modulation could be mitigated by other homologous factors, analysis of LIF-null or LIF-modulated organisms would be the best way to determine the true extent to which LIF modulation affects non-reproductive systems in-vivo. Additionally, given that Menkhorst *et al.* showed systemic PEGLA administration altered bone density, researchers who aim to develop novel non-hormonal contraceptives should move away from systemic LIF modulation and towards uterus-targeted LIF modulation via vaginal administration (9) as a more cautious approach.

Hormonal Contraceptives

A discussion of the need for and merits of LIF modulation as a non-hormonal alternative to current contraception necessitates a brief overview of hormonal contraceptives: their formulation, basic mechanism of action, and adverse effects. This will help elucidate whether or not the development of non-hormonal contraceptives in general, and LIF modulators specifically, can bring about more social benefit than research regarding the amelioration of hormonal contraceptives.

While various different compositions and forms of hormonal contraceptives are presently available, the most common are combined oral contraceptives (COC). As such, they are a reasonable standard against which novel contraceptives such as LIF antagonists can be compared. Given that COC is used by over 100 million women worldwide as a form of birth control, its associated adverse side effects should be viewed as an important public health issue. (31)

Combined oral contraceptives pills contain both synthetic estrogen (typically containing synthetic ethynyl derivatives, ethinyl-estradiol and mestranol) and synthetic progesterone (typically containing synthetic desogestrel, ethynodiol diacetate, gestodene, levonorgestrel, lynestrenol, norethisterone, norethisterone acetate, norgestimate or norgestrel). (8) The progesterone and estrogen analogs inhibit LH and FSH peaks, which normally occur prior to ovulation, via negative feedback. Inhibition of the LH surge stops ovulation, or the release of an egg from the ovaries into the peritoneal cavity. Further, the progestin component thickens cervical mucus, which decreases sperm penetration, and reduces endometrial proliferation, which decreases uterine receptivity to implantation. Most combined oral contraceptives contain placebo pills without estrogen or progestin to stimulate menstrual bleeding while keeping women in the habit of taking the pill every day.

Beyond combined oral contraceptives containing both synthetic estrogen and progestins, progestin-only forms of contraception are also widely available. (32) Progestin-only contraceptives are often prescribed when estrogen administration is problematic. For example, exogenous estrogen is thought to negatively affect milk production in breastfeeding mothers (8,33).

While hormonal contraceptives are effective in preventing pregnancy, their use has been associated with an increased risk of thromboembolism, hypertension, and gallstones. (8) Both combined oral hormonal contraceptives (COC) and progestin-only contraceptives (POC) are associated with altered bone metabolism. Decreased bone turnover is observed in both formulations of OC, while decreased bone resorption is observed only in POC. (43) Further, COC use is associated with a small, yet significant increased risk of breast cancer. However, COC use has been associated with a decreased risk of endometrial, ovarian and colorectal cancer. (34)

Aside from physical ailments, a postulated side effect of hormonal oral contraceptive use is depression or dysthymia. Yet, studies regarding the extent of this effect have been inconsistent; some studies find that birth control is not associated with worsened effect (36,37), while others show the opposite. (8,38) Skovlund *et al.* found that COC users were 1.8X more likely that non-users to start using antidepressants for the first time. (38) This trend was even more pronounced for POC users, who were 2.2X more likely to start antidepressant use. Inconsistency in this research may be due to varied contraceptive formulations and approaches to measuring depressive symptoms. Further, the multifactorial nature of depression complicates studies. (37)

Controversy notwithstanding, estrogen and progesterone have been shown to have psychoactive properties. (39,40) The precise mechanism of and extent to which estrogen and progesterone lead to depressive symptoms is still unclear. (8,41) Current theories generally attribute depressive symptoms to progestins. (37) For example, progesterone is postulated to decrease serotonin levels by increasing the activity of monoamine oxidase, which degrades serotonin[5]). (8,38) Progesterone metabolites have also been shown to act on the γ-aminobutyric acid A (GABA) receptor complex, a major inhibitory system in the human CNS. (38) As follows, various studies have also shown that oral contraceptives with high progestin

doses are more frequently associated with depressive symptoms. (41,38) For instance, Lawrie et al found that women taking progestin-containing injectable contraceptives postpartum scored significantly higher than the placebo group in terms of depressive symptoms as measured by the Montgomery-Asberg Depression Rating Scale. (41)

Vaginal administration of synthetic estrogens and progestins via a vaginal ring (somewhat analogous to vaginal PEGLA administration (9)), may alter the severity or incidence of negative side effects associated with combined oral contraceptive use in women. Select studies have shown that ring usage is associated with a lower incidence of negative COC-associated side effects, such as depression. Unfortunately, ring-usage is also associated with increased incidence and severity of local side effects such as leukorrhea and vaginitis. (42)

Conclusion/Future Directions

The development of novel non-hormonal birth-control methods is imperative as hormonal contraceptives have been associated with an increased risk of depression (8,38) and are not suitable for women with certain pre-existing conditions. (44) LIF modulation could potentially be effective for this purpose.

However, one should be wary of the effects of systemic LIF modulation, as LIF is involved in many non-reproductive systems. No studies to date have fully characterized the effects of systemic LIF modulation in mice, primates, or humans. Systemic inhibition of LIF activity, via LIF-antagonists or immunization, could therefore have adverse side effects. Future studies should aim to better characterize non-reproductive health (bone density, blood composition and myelin integrity) in mice or primates receiving systemic or local inhibition of LIF, and in LIF-null mice. This would allow researchers to better understand the potential side effects of LIF modulation, and likely further support the stipulation made by Menkhorst *et al.* (9) that systemic LIF-modulation may not be appropriate for contraceptive purposes.

Menkhorst *et al.* demonstrate that adverse side effects of systemic LIF-modulation, such as altered bone density, could be mitigated by administering PEGLA vaginally and thus, focusing its LIF-inhibitory activity to the uterus. (9) They also propose that a vaginal gel delivering PEGLA could also be used to simultaneously deliver microbicides to inhibit sexually-transmitted infections (STIs). (9) However, much remains to be done before such a gel can be developed. In addition to performing further animal studies, research should be performed to determine if women would actually want to use a gel as their primary contraceptive method, or if it seems too messy or difficult to administer. If the latter were the case, PEGLA could potentially be delivered via a vaginal ring.

Further, more research ought to be done regarding the adverse side effects associated with hormonal birth control usage. Specifically, given that previous research regarding depression and oral contraceptive usage has yielded inconsistent results and sparked disagreement amongst scientists (37,38), further meta-analysis of human data as was done by Skovlund *et al.* ought to be done to conclusively demonstrate that COC usage increases the risk of depression. Further, more direct methods should be employed in mouse models to try and understand the molecular mechanism of this observed effect.

A deeper understanding of the mechanism by which hormonal contraceptives bring about adverse side effects will serve as a good starting point for remodulating these drugs. For instance, some studies have shown that the depressive side effects of COC are mostly due to the progestin component (37), so future formulations might be improved by simply decreasing the amount of progestin. Remodulated oral contraceptives could potentially decrease the severity of negative side effects and would likely be publicly available much sooner than LIF-modulating contraceptives which are still in the preliminary stages of development.

Alternatively, a better characterization of the negative effects of hormonal contraceptives could further support the argument that society ought to invest heavily in the development of novel, non-hormonal contraceptives, namely PEGLA. Present studies suggest that vaginal PEGLA administration could be associated with fewer negative side effects than COC.(9) Additionally, several non-LIF genes have been identified in the uterus which are also vital for maintaining pregnancy. (2) Like LIF, these genes could potentially be modulated for contraceptive purposes.

To conclude, present research suggests that vaginally administered PEGLA could be as effective as hormonal contraceptives in terms of preventing pregnancy in women. (7,9,21) While systemic LIF-modulation has been shown to be associated with altered bone density, vaginal administration of PEGLA seems to mitigate this risk without enhancing local side effects, which was the case when hormonal contraceptives were administered vaginally (9,42) Therefore, in terms of mitigating negative side effects and thereby increasing clinical desirability, vaginally administered LIF-modulating contraceptives could be preferable to hormonal contraceptives.

[1] Decidualization is the process by which stromal cells differentiate, resulting in the formation of a "decidua", which provides the blastocyst with nutrients until the functional placenta is formed, while simultaneously restraining trophoblast-uterine invasion. (4,5)

[2] A secondary, small ovarian estrogen pulse, occurring on GE day 4 in mice.

[3] A biochemical technique that identifies vital residues of a studied protein by replacing single residues with alanine, via targeted mutagenesis, and subsequently assaying protein function.

[4] Organic component of bone.

[5] Monoamine oxidase inhibitors are a form of antidepressant. (39)

References

1. Cross JC, Werb Z, Fisher SJ. Implantation and the placenta: key pieces of the development puzzle. Science. 1994 Dec 2;266(5190):1508–18.

2. Kelleher AM, Milano-Foster J, Behura SK, Spencer TE. Uterine glands coordinate on-time embryo implantation and impact endometrial decidualization for pregnancy success. Nature Communications. 2018 Jun 22;9(1):1–12.

3. 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 Sep 3;359(6390):76–9.

4. Okada H, Tsuzuki T, Murata H. Decidualization of the human endometrium. Reprod Med Biol. 2018 Feb 1;17(3):220–7.

5. Rosario GX, Stewart CL. The Multifaceted Actions of Leukaemia Inhibitory Factor in Mediating Uterine Receptivity and Embryo Implantation. Am J Reprod Immunol. 2016 Mar;75(3):246–55.

6. Lemons AR, Naz RK. Birth control vaccine targeting leukemia inhibitory factor. Molecular Reproduction and Development. 2012;79(2):97–106.

7. Lalitkumar S, Boggavarapu NR, Menezes J, Dimitriadis E, Zhang J-G, Nicola NA, et al. Polyethylene glycated leukemia inhibitory factor antagonist inhibits human blastocyst implantation and triggers apoptosis by down-regulating embryonic AKT. Fertility and Sterility. 2013 Oct 1;100(4):1160-1169.e2.

8. Kulkarni J. Depression as a side effect of the contraceptive pill. Expert Opin Drug Saf. 2007 Jul;6(4):371–4.

9. Menkhorst E, Zhang J-G, Sims NA, Morgan PO, Soo P, Poulton IJ, et

al. Vaginally Administered PEGylated LIF Antagonist Blocked Embryo Implantation and Eliminated Non-Target Effects on Bone in Mice. PLOS ONE. 2011 May 18;6(5):e19665.

10. Gray CA, Bartol FF, Tarleton BJ, Wiley AA, Johnson GA, Bazer FW, et al. Developmental biology of uterine glands. Biol Reprod. 2001 Nov;65(5):1311–23.

11. Spencer TE. Biological Roles of Uterine Glands in Pregnancy. Semin Reprod Med. 2014 Sep;32(5):346–57.

12. 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 Sep 3;359(6390):76–9.

13. Wu M, Yin Y, Zhao M, Hu L, Chen Q. The low expression of leukemia inhibitory factor in endometrium: possible relevant to unexplained infertility with multiple implantation failures. Cytokine. 2013 May;62(2):334–9.

14. Zhang W, Kim SM, Wang W, Cai C, Feng Y, Kong W, et al. Cochlear Gene Therapy for Sensorineural Hearing Loss: Current Status and Major Remaining Hurdles for Translational Success. Front Mol Neurosci [Internet]. 2018 Jun 26 [cited 2019 Nov 18];11. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6028713/

15. Serafini PC, Silva ID, Smith GD, Motta EL, Rocha AM, Baracat EC. Endometrial claudin-4 and leukemia inhibitory factor are associated with assisted reproduction outcome. Reprod Biol Endocrinol. 2009 Apr 19;7:30.

16. Chen JR, Cheng JG, Shatzer T, Sewell L, Hernandez L, Stewart CL. Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. Endocrinology. 2000 Dec;141(12):4365–72.

17. Salleh N, Giribabu N. Leukemia Inhibitory Factor: Roles in Embryo Implantation and in Nonhormonal Contraception [Internet]. The Scientific World Journal. 2014 [cited 2020 Jan 17]. Available from: https://new.hindawi.com/journals/tswj/2014/201514/

18. Stewart CL. Leukaemia inhibitory factor and the regulation of pre-implantation development of the mammalian embryo. Mol Reprod Dev. 1994 Oct;39(2):233–8.

19. Dimitriadis E, Menkhorst E. New generation contraceptives: interleukin 11 family cytokines as non-steroidal contraceptive targets. J Reprod Immunol. 2011 Mar;88(2):233–9.

20. Fairlie WD, Uboldi AD, McCoubrie JE, Wang CC, Lee EF, Yao S, et al. Affinity maturation of leukemia inhibitory factor and conversion to potent antagonists of signaling. J Biol Chem. 2004 Jan 16;279(3):2125–34.

21. White CA, Zhang J-G, Salamonsen LA, Baca M, Fairlie WD, Metcalf D, et al. Blocking LIF action in the uterus by using a PEGylated antagonist prevents implantation: a nonhormonal contraceptive strategy. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(49):19357–62.

22.Ernst M, Inglese M, Waring P, Campbell IK, Bao S, Clay FJ, et al. Defective gp130-mediated signal transducer and activator of transcription (STAT) signaling results in degenerative joint disease, gastrointestinal ulceration, and failure of uterine implantation. J Exp Med. 2001 Jul 16;194(2):189–203.

23. Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci U S A. 1996 Apr 2;93(7):3115–20.

24. Reddy KR, Wright TL, Pockros PJ, Shiffman M, Everson G, Reindollar R, et al. Efficacy and safety of pegylated (40-kd) interferon alpha-2a compared with interferon alpha-2a in noncirrhotic patients with chronic hepatitis C. Hepatology. 2001 Feb;33(2):433–8.

25. Sengupta J, Lalitkumar PGL, Najwa AR, Ghosh D. Monoclonal anti-leukemia inhibitory factor antibody inhibits blastocyst implantation in the rhesus monkey. Contraception. 2006 Nov;74(5):419–25.

26. Estrov Z, Talpaz M, Wetzler M, Kurzrock R. The modulatory hematopoietic activities of leukemia inhibitory factor. Leukemia & Lymphoma. 1992 Jan 1;8(1–2):1–7.

27. Hudson KR, Vernallis AB, Heath JK. Characterization of the Receptor Binding Sites of Human Leukemia Inhibitory Factor and Creation of Antagonists. J Biol Chem. 1996 May 17;271(20):11971–8.

28. Butzkueven H, Zhang J-G, Soilu-Hanninen M, Hochrein H, Chionh F, Shipham KA, et al. LIF receptor signaling limits immune-mediated demyelination by enhancing oligodendrocyte survival. Nat Med. 2002 Jun;8(6):613–9.

29. Martin TJ, Seeman E. New mechanisms and targets in the treatment of bone fragility. Clin Sci. 2007 Jan;112(2):77–91.

30. Cicinelli E, de Ziegler D. Transvaginal progesterone: evidence for a new functional "portal system" flowing from the vagina to the uterus. Hum Reprod Update. 1999 Aug;5(4):365–72.

31. Christin-Maitre S. History of oral contraceptive drugs and their use worldwide. Best Pract Res Clin Endocrinol Metab. 2013 Feb;27(1):3–12.

32. Young EA, Kornstein SG, Harvey AT, Wisniewski SR, Barkin J, Fava M, et al. Influences of hormone-based contraception on depressive symptoms in premenopausal women with major depression. Psychoneuroendocrinology. 2007 Aug;32(7):843–53.

33. Kelsey JJ. Hormonal contraception and lactation. J Hum Lact. 1996 Dec;12(4):315–8.

34. Schneyer R, Lerma K. Health outcomes associated with use of hormonal contraception: breast cancer. Curr Opin Obstet Gynecol. 2018;30(6):414–8.

35. Robinson SA, Dowell M, Pedulla D, McCauley L. Do the emotional side-effects of hormonal contraceptives come from pharmacologic or psychological mechanisms? Med Hypotheses. 2004;63(2):268–73.

36. Oinonen KA, Mazmanian D. To what extent do oral contraceptives influence mood and affect? J Affect Disord. 2002 Aug;70(3):229–40.

37. Bitzer J. Hormonal contraception and depression: another Pill scandal? Eur J Contracept Reprod Health Care. 2017;22(1):1–2.

38. Skovlund CW, Mørch LS, Kessing LV, Lidegaard Ø. Association of Hormonal Contraception With Depression. JAMA Psychiatry. 2016 Nov 1;73(11):1154–62.

39.Biegon A, Reches A, Snyder L, McEwen BS. Serotonergic and noradrenergic receptors in the rat brain: modulation by chronic exposure to ovarian hormones. Life Sci. 1983 Apr 25;32(17):2015–21.

40. Toffoletto S, Lanzenberger R, Gingnell M, Sundström-Poromaa I, Comasco E. Emotional and cognitive functional imaging of estrogen and progesterone effects in the female human brain: a systematic review. Psychoneuroendocrinology. 2014 Dec;50:28–52.

41. Lawrie TA, Hofmeyr GJ, Jager MD, Berk M, Paiker J, Viljoen E. A double-blind randomised placebo controlled trial of postnatal norethisterone enanthate: the effect on postnatal depression and serum hormones. BJOG: An International Journal of Obstetrics & Gynaecology. 1998;105(10):1082–90.

42. Lete I, Arrilucea MP de, Rodríguez M, Bello E. Efficacy, safety, and patient acceptability of the etonogestrel and ethinyl estradiol vaginal ring

[Internet]. Open Access Journal of Contraception. 2014 [cited 2020 Jan 17].

43. Herrmann M, Seibel The effects of hormonal contraceptives on bone turnover markers and bone health Clinical Endocrinology (2010) 72, 571–583 doi 10.1111/j.1365-2265.2009.03688.x

44. ACOG Practice Bulletin No. 206. Obstetrics & Gynecology [Internet]. 2019 Feb [cited 2020 Mar 4];133(2):e128–50. Available from: https://journals.lww.com/greenjournal/Fulltext/2019/02000/ACOG_Practice_Bulletin_No__206__Use_of_Hormonal.41.aspx