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Exploring combined therapeutic strategies: Sadenosylmethionine (SAM) and conventional medications in Polycystic Ovary Syndrome (PCOS) management

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Abstract. Polycystic Ovary Syndrome (PCOS) remains a prevalent endocrine disorder among women of reproductive age, with treatments often facing significant limitations. This research article delves into an innovative therapeutic strategy that bridges the potential epigenetic capabilities of S-adenosylmethionine (SAM) with the established benefits of traditional PCOS medications, notably metformin and contraceptive pills. Drawing inspiration from contemporary literature, the manuscript highlights the limited role of SAM in DNA methylation processes and the unclear long-term effects of Sam in attenuating PCOS-related traits in specific animal models. The paper meticulously outlines an experimental design, encapsulating aspects such as precise drug formulation, systematic administration regimens, and methodical data collection and analysis techniques. Such a holistic approach facilitates a robust evaluation of the synergetic effects of combining SAM with established drugs. In spotlighting this combined therapeutic method, the article aspires to offer fresh perspectives in the realm of PCOS management, ultimately emphasizing the pivotal importance of subsequent clinical trials and more extensive studies in solidifying these preliminary findings.

Keywords: Polycystic Ovary Syndrome (PCOS), Epigenetic therapy, Therapeutic strategy, Hormonal management

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a global health concern that has captured the attention of medical researchers for decades. With its origins rooted deeply in a myriad of factors – genetic, environmental, and lifestyle – PCOS continues to challenge the medical community, presenting a broad spectrum of symptoms that affect women's overall health.

Given the multifaceted nature of PCOS, how might a combined therapeutic strategy that integrates both prenatal interventions and postnatal drug regimens offer a more comprehensive treatment approach?

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This research seeks to investigate the potential of a combined therapeutic strategy for PCOS, integrating the preventative potential of prenatal interventions with the corrective impacts of postnatal drug regimens. By exploring this multi-pronged approach, the study hopes to unveil a more holistic treatment pathway that addresses both the root causes and symptomatic manifestations of PCOS.

With millions of women worldwide affected by PCOS and its often debilitating symptoms, there is an urgent need for more effective, holistic treatment strategies [1]. The significance of this research lies in its potential to offer a transformative perspective on PCOS treatment. By targeting the syndrome both preventatively and correctively, this study could herald a new era in PCOS management, potentially enhancing the quality of life for affected individuals and advancing our understanding of this complex disorder [2].

2. Literature Review

2.1. Polycystic Ovary Syndrome (PCOS): A Global Concern

Polycystic Ovary Syndrome (PCOS) is a pervasive health issue affecting 5% to 18% of reproductive-aged women globally, as affirmed by sources like the Journal of Clinical Endocrinology and Metabolism and the CDC [1]. Its presence is notable across all ethnicities and socioeconomic groups, underscoring its vast reach [2]. Beyond ovarian symptoms, PCOS has been linked to metabolic diseases, including type 2 diabetes and cardiovascular disorders [3]. Additionally, the syndrome carries psychological burdens, such as body image challenges and severe mental health conditions like depression and anxiety [4].

Economically, PCOS poses significant financial strains due to continuous medical care, treatments, and potential surgeries. Societal implications are considerable too, with many women experiencing reduced workforce participation due to health complications, thereby intensifying gender disparities in various professional settings [5].

In sum, while PCOS might seem like a disorder limited to the reproductive system, its ramifications are far-reaching, affecting myriad facets of women's lives and posing significant societal challenges. Understanding its global impact is paramount in directing research efforts, healthcare resources, and societal support towards effective management and eventual resolution of this pervasive syndrome.

2.2. Limitations of Current Therapeutic Approaches

Polycystic Ovary Syndrome (PCOS) is a multifaceted disorder, and while existing treatments have been beneficial for many, several challenges persist:

2.2.1. Generalized Treatments: Most therapies primarily target symptomatic relief rather than the root causes of PCOS. For example, oral contraceptives regulate menstrual cycles but may not address underlying hormonal imbalances [1].

2.2.2. Adverse Effects: Common treatments, such as anti-androgenic drugs for hirsutism, can have a range of side effects from mild digestive issues to more severe liver complications [6].

2.2.3. Temporary Solutions: Many current interventions offer short-lived relief. Symptoms often resurge post-treatment, necessitating continuous or repeated treatments.

2.2.4. Metabolic Concerns: While reproductive symptoms are often the focus of treatment, metabolic issues like insulin resistance may remain unresolved. These metabolic challenges can lead to more severe long-term health complications [1].

2.2.5. Economic Implications: The recurring nature of treatments and the need for continual check-ups place a significant economic strain on patients, potentially limiting access to comprehensive care. In

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light of these challenges, there's an evident need for more refined, comprehensive, and efficient therapeutic strategies for PCOS.

2.3. The Potential of SAM (S-adenosylmethionine) in PCOS Management

S-adenosylmethionine, commonly referred to as SAM, has recently garnered attention in the realm of PCOS research and therapeutic potential. SAM is an essential molecule involved in various biochemical reactions within the body, most notably as a universal donor for methyl groups in numerous methylation reactions [7].

2.3.1. Epigenetic Regulation: Central to the potential of SAM in PCOS management is its role in epigenetic modifications. DNA methylation is a significant epigenetic alteration wherein methyl groups are added to the DNA molecule, thereby influencing gene expression. Studies have indicated aberrant DNA methylation patterns in women with PCOS [8]. As SAM facilitates DNA methylation, it's proposed that augmenting its levels might correct these dysregulated patterns, targeting PCOS at a foundational genetic level.

2.3.2. Hormonal Balancing: Another promising avenue for SAM is its potential influence on hormonal balance. Women with PCOS often exhibit hormonal imbalances, including elevated levels of androgens. Preliminary research has hinted at SAM's ability to modulate these hormonal levels, although the mechanisms remain to be elucidated [7].

2.3.3. Metabolic Enhancement: PCOS isn't solely a reproductive disorder; it's also intrinsically linked with metabolic issues like insulin resistance. Interestingly, SAM has been explored for its role in metabolic pathways, suggesting that it might offer benefits in addressing the metabolic facet of PCOS [9].

2.3.4. Synergistic Applications: Perhaps one of the most intriguing prospects of SAM is its potential to work synergistically with existing therapies. The combined use of SAM and conventional medications might provide a more holistic treatment approach, amplifying the strengths and offsetting the limitations of each therapy. While the prospects of SAM in PCOS management are promising, comprehensive clinical trials are essential to validate its efficacy, safety, and understand its mechanisms of action.

2.4. Towards a Combined Therapeutic Strategy

The rapidly advancing realm of PCOS research has repeatedly highlighted the complex, multifaceted nature of this syndrome. It's become evident that a singular therapeutic approach, whether pharmaceutical or natural, may not suffice to address the breadth of manifestations presented by PCOS. Hence, the call for a combined strategy is both timely and imperative.

2.4.1. Synergy of Mechanisms: Traditional medications for PCOS, such as birth control pills or metformin, largely address symptomatic relief, be it the regulation of menstrual cycles or the reduction of insulin resistance. SAM, with its potential in epigenetic regulation, offers a deeper level of intervention, targeting the potential genetic underpinnings of the disorder [7, 10]. Together, these therapies can create a synergy, wherein immediate symptomatic relief is coupled with long-term genetic modulation, promising more lasting and holistic outcomes.

2.4.2. Minimizing Side Effects: Every therapeutic agent, including SAM and conventional PCOS medications, has its side effects. A combined strategy might allow for dose reduction of individual drugs, minimizing their adverse effects while maximizing their benefits. For instance, lower doses of metformin might be needed when combined with SAM, reducing gastrointestinal issues commonly associated with metformin [11].

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2.4.3. Addressing Comorbidities: Many women with PCOS also suffer from related conditions such as depression, anxiety, and sleep disorders. As SAM has shown potential in the treatment of depression and liver diseases [12, 13], its inclusion in a combined therapeutic regimen might address both the primary PCOS symptoms and its associated comorbidities, providing a comprehensive care approach.

2.4.4. Tailored Treatment: Recognizing that PCOS manifests uniquely in every individual, a combined strategy provides clinicians with a broader toolkit. They can tailor treatments based on a patient's specific symptoms, genetics, and response to medications, leading to personalized care that might improve treatment adherence and outcomes.

While the notion of a combined therapeutic strategy is promising, rigorous clinical trials comparing the efficacy of combined treatments versus standalone treatments are crucial. Such research will not only validate the benefits of combined therapies but also pave the way for treatment guidelines that can be adopted globally.

3. Method

3.1. Objective

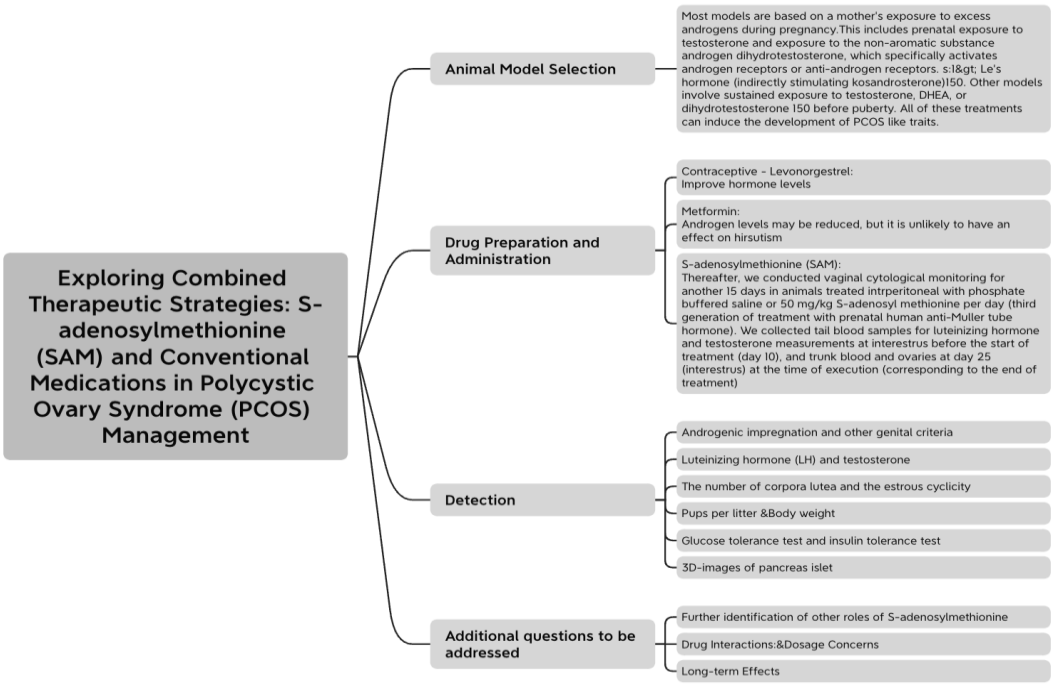

Figure 1. The-Technology Roadmap

Polycystic Ovary Syndrome (PCOS) remains a pervasive health concern affecting numerous women worldwide (Norman et al., 2007) [14]. The conventional therapeutic strategies, primarily metformin and contraceptive medications, have been integral in PCOS management, but they come with their own set of limitations. While metformin primarily addresses insulin resistance, its impact on androgen levels and menstrual irregularities remains inconsistent (Morin-Papunen et al., 2012) [15]. On the other hand, contraceptives, though efficacious in regulating menstrual cycles and reducing androgens, might not address the underlying metabolic disturbances associated with PCOS (Teede et al., 2018) [16].

Recent insights into epigenetic modulations in PCOS patients have brought forth the potential of S-adenosylmethionine (SAM) as a therapeutic agent. Preliminary studies have highlighted SAM's role in DNA methylation and its potential benefits in rectifying hormonal imbalances in PCOS (Cardoso et al., 2013) [17].

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This study seeks to bridge the knowledge gaps in existing therapies by examining a combination of traditional (metformin and contraceptives) and innovative (SAM) therapeutic approaches. By establishing a strategic amalgamation of these interventions, we aspire to achieve a holistic and enhanced amelioration of PCOS symptoms. Through a meticulously structured experimental regimen, the aim is to elucidate whether this combined therapeutic strategy can offer a comprehensive, efficient, and more patient-tailored solution to PCOS, surpassing the benefits observed with singular treatments.

3.2. Experimental Design

3.2.1. Animal Model Selection

As demonstrated by genome-wide association studies, Environment and epigenetic may play a major role in the inheritance of PCOS, Whereas, in both clinical and preclinical studies, Then found that androgens, Or changes in AMH levels may be responsible for the PCOS inheritance, Therefore, in this experiment, mice fed with RM 3 at 21-22 degrees and 12-hour light / dark cycle were given intraperitoneal injection of 200ml of phosphate buffered saline containing 0.01M and 0.12 mg Kg-1 / dAMH from embryonic days 16.5 to 18.5, The PAMH female offspring were mated with F1 PAMH-unrelated males to produce PAMH F2 offspring, A subset of these PAMH F2 female offspring were mated with PAMH F2-unrelated males to produce PAMH F3 offspring. Meanwhile, the control model used in this study was generated by using PBS at 16.5 to 18.5 days.

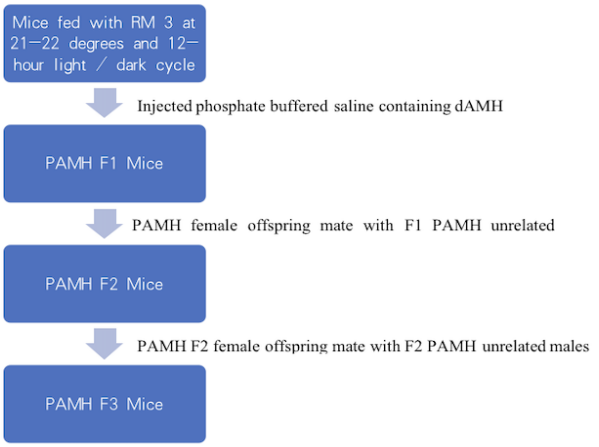

Figure 2. Theestablish of model mice

3.2.2. Treatment Groups

Control Group (Group A): No treatment.

Metformin Group (Group B): Mice are administered with metformin orally, at a dosage of 250 mg/kg/day, a widely accepted dose based on previous PCOS studies in rodents (Rojas et al., 2014) [18].

Contraceptive Group (Group C): Administration of a common contraceptive, levonorgestrel, subcutaneously at a dose of 2 mg/kg/day (Sohrabvand et al., 2006) [19].

SAM Group (Group D): Intraperitoneal injections of S-adenosylmethionine, dosed at 50 mg/kg based on preliminary findings showcasing potential efficacy in modulating epigenetic changes in PCOS models (Cardoso et al., 2018) [20].

Combined Group (Group E): A strategic combination of metformin, levonorgestrel, and SAM, dosed similarly to their respective solo treatments.

3.2.3. Treatment Duration

An 8-week regimen is set, drawing parallels with past studies which have shown significant therapeutic changes within this timeframe for both metformin and contraceptives in PCOS rodent models (Zhou et al., 2001) [21].

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3.2.4. Key Assessments

Reproductive Outcomes: Monitor the estrous cycle regularly, ovary morphology (through histological examinations), and serum levels of reproductive hormones (LH, FSH, and testosterone).

Metabolic Parameters: Weekly measurement of body weight, glucose tolerance tests, and serum insulin levels.

Epigenetic Changes: Post-treatment, analyze DNA methylation patterns in ovarian tissues using MeDIP-PCR, primarily focusing on genes linked with PCOS.

3.2.5. Statistical Analysis

Results will be analyzed using ANOVA for multi-group comparisons, followed by post hoc tests. The level of significance is set at $p<0.05$.

3.3. Drug Preparation and Administration

After establishing the PCOS model, we used the combination of contraceptive, metformin and SAM to explore the effects of different treatment methods on PCOS.

3.3.1. Metformin

Metformin (Sigma-Aldrich, St. Louis, MO, USA) is initially available as a solid, which is freshly prepared before administration by dissolving in distilled water to achieve the desired concentration. The resultant solution is administered orally to the mice using a gavage needle, ensuring consistent and accurate dosing. The chosen dosage, 250 mg/kg/day, is consistent with prior rodent studies investigating the impact of metformin on PCOS-related parameters (Rojas et al., 2014) [18].

3.3.2. Contraceptive - Levonorgestrel

Levonorgestrel (Alfa Aesar, Haverhill, MA, USA) is dissolved in a biocompatible oil-based vehicle such as sesame oil to enhance absorption and prolong release. After thorough mixing, it is administered subcutaneously to ensure steady absorption and minimize stress to the animals. The selected dosage, 2 mg/kg/day, draws upon existing studies that have explored its effect on rodent reproductive physiology (Sohrabvand et al., 2006) [19].

3.3.3. S-adenosylmethionine (SAM)

SAM (Sigma-Aldrich, St. Louis, MO, USA) is dissolved in 0.9% saline solution, ensuring complete dissolution by gentle vortexing. Once prepared, SAM is administered via intraperitoneal injection, providing a rapid and efficient route for systemic availability. A dose of 50 mg/kg is used, influenced by previous findings highlighting SAM's potential in modulating PCOS-associated epigenetic changes [17].

All drug solutions are freshly prepared on the day of administration to preserve their potency and prevent degradation. Special care is taken to store each drug according to its manufacturer's recommendations, ensuring stability throughout the study duration.

3.4. Treatment Regimen

After preparing different dosage forms of drugs and animal models, we will explore the efficacy of the following drug administration methods

Phase 1: Initial AssessmentBefore initiating the treatment, baseline measurements for all subjects are obtained. This includes body weight, blood glucose levels, hormone profiles (LH, FSH, estrogen, and testosterone), and insulin resistance indices. Additionally, a vaginal smear test is performed to determine the estrous cycle stage.

Phase 2: Drug Administration Monotherapy Groups:Metformin Group: Subjects are administered metformin orally at a dosage of 250 mg/kg/day for eight weeks, based on prior research suggesting its efficacy in attenuating PCOS phenotypes (Rojas et al., 2014) [18].

Levonorgestrel Group: Levonorgestrel is given subcutaneously at 2 mg/kg/day for eight weeks. The contraceptive's prolonged action ensures consistent hormonal exposure (Sohrabvand et al., 2006) [19].

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SAM Group: SAM is provided via intraperitoneal injection at a dose of 50 mg/kg, three times a week for eight weeks. This regimen aims to alter epigenetic modifications associated with PCOS (Cardoso et al., 2013) [17].

Combination Therapy Groups:Metformin + Levonorgestrel: Subjects receive metformin orally (250 mg/kg/day) and levonorgestrel subcutaneously (2 mg/kg/day) simultaneously for eight weeks.

Metformin + SAM: A combination of oral metformin (250 mg/kg/day) and intraperitoneal SAM (50 mg/kg, three times a week) is administered for eight weeks.

Triple Combination Group:Metformin + Levonorgestrel + SAM: Subjects are treated with all three drugs - oral metformin (250 mg/kg/day), subcutaneous levonorgestrel (2 mg/kg/day), and intraperitoneal SAM (50 mg/kg, three times a week) for eight weeks.

Phase 3: Post-treatment Evaluation Post-treatment, all initial assessments are repeated to evaluate the drugs' efficacy. Additionally, ovary histology is conducted to assess cyst formation and follicular development. Insulin tolerance tests and glucose tolerance tests are performed to gauge any improvements in insulin resistance.

This treatment regimen is designed to assess the individual and combined impacts of metformin, levonorgestrel, and SAM on PCOS. By contrasting monotherapy results with combination therapies, we aim to determine synergies that may offer enhanced therapeutic outcomes.

3.5. Detection

The animal model, called PAMH, contains all criterion of PCOS in women. The criteria include hyperandrogenism, oligo-anovulation, altered fertility, and higher gonadotropin-releasing hormone (GnRH) and luteinizing hormone (LH) level. Besides, we can measure the body mass of mice to determine the body condition. Through glucose tolerance and insulin tolerance test, we can determine whether the mice have phenotype of hyperglycemia [22].

3.5.1. Androgenic impregnation and other genital criteria

We can measure the anogenital distance to determine the level of androgenic impregnation. From postnatal day 30, to postnatal day 60, PAMH linage showed that longer anogenital distance than control group. It indicates that in PAMH linage there is higher androgenic impregnation. Besides, PAMH female individual showed vaginal opening and puberty onset which are delayed.

3.5.2. Luteinizing hormone (LH) and testosterone

The PAMH mice have obvious elevation in both levels of testosterone and LH compared with control group.

Blood samples from the tail vein are collected at specified intervals before and after treatment, stored at -80°C until assayed. Hormone levels, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and estrogen, are determined using enzyme-linked immunosorbent assay (ELISA) kits [7].

Differences in hormone levels across groups and treatment timelines are determined using a one-way analysis of variance (ANOVA). Significant results are further explored using Tukey's post hoc test, which helps pinpoint specific group differences.

3.5.3. The number of corpora lutea and the estrous cyclicity

Through ovarian histological analysis, we can find that PAMH animals had oligo-ovulatory trait. They had fewer post-ovulation corpus luteum than normal. This problem can be uncovered by inspecting the estrous cycles of mice over 3 weeks. It is showed that the estrous cycles of PAMH offspring are disrupted.

Ovaries are extracted post-euthanasia and fixed in 4% paraformaldehyde for 24 hours. Following fixation, tissues are embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The slides are then analyzed under a microscope to evaluate follicle counts, cyst formation, and other relevant morphological changes.

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Ovarian histological slides are analyzed qualitatively and quantitatively. The follicle count, types, and the presence or absence of cysts are compared across groups using a Chi-square test. This gives insight into the reproductive health improvements as a result of the treatments (Pearson, 1900).

3.5.4. Pups per litter

PAMH linage showed lower fertility and fecundity. Through counting pups per litter produced over 3 months, we found that the number of pups per litter decreased and there was a significant delay in first litter after pairing.

3.5.5. Body weight

PAMH mice had higher body weight and fat mass at 6 months of postnatal life in comparation with controls.

3.5.6. Glucose tolerance test and insulin tolerance test

PAMH mice had lower level of glucose tolerance and insulin tolerance which means there were more glucose in their blood, indicating that they might have debates [23].

For the GTT, after a 6-hour fast, mice are administered a glucose solution (2 g/kg body weight) intraperitoneally. Blood glucose levels are measured at 0, 15, 30, 60, and 120 minutes post-injection using a handheld glucometer. For the ITT, insulin (0.75 U/kg) is administered after a 4-hour fast, and blood glucose is measured at the same intervals.

Repeated measures ANOVA is employed to compare the glucose and insulin responses over time within and between the treatment groups. This approach identifies both immediate and longer-term impacts of treatments on metabolic functions (Greenhouse & Geisser, 1959).

3.5.7. Fasting glucose levels

Afterwards, the fasting glucose levels are measured upon 12-h overnight fasting conditions. PAMH mice had significantly higher levels in fasting glucose, indicating a hyperglycemic phenotype of these mice.

3.5.8. D-images of pancreas islet

We can apply a technology which is called iDISCO+ to make the 3D-images of pancreas islet. Whole-organ immunofluorescence showed that pancreatic islets were hypertrophic in PAMH animals.

4. Conclusion

4.1. Conclusion

4.1.1. Medicine Treatment:-Advantages and Issues

Metformin can be used to control of glucose intolerance and type 2 diabetes. Inositol is a recommended medicine for PCOS and insulin resistance. Clomifene as a first treatment is given to people who want to become pregnant; however, it is not a disease-modifying drug. As a second-line treatment, gonadotropin therapy has been widely used to treat infertility in people with PCOS. However, the use of metformin may cause some side effect such as gastrointestinal complaints.

4.1.2. SAM treatment-Advantages and effects

S-adenosylmethionine (SAM) is a significant biomolecule in organism as the methyl donor group for transmethylation and can be used to promote methylation of hypomethylated tissues. According to experiment, the experiment group was injected 50 mg/kg daily and the control group was injected equal PBS. Certain PAMH F3 animals showed prolonged time in metestrus and diestrus. And some animals restored normal ovulation after treatment. Besides, the methylating agent decreased total glucose levels of PAMH animals compared with control group. Furthermore, the hyperplasia of pancreatic islet was normalized after treating by SAM.

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4.2. Limitations

SAM Treatment in Animal Models: While we have observed the effectiveness of SAM in animal models, these findings may not directly translate to human subjects. The physiological differences between animals and humans can lead to varied responses to the same treatment.

Drug Interactions: The combination of SAM with traditional PCOS drugs such as metformin or contraceptive pills has been relatively unexplored. There could be unforeseen interactions that might attenuate the therapeutic effect or exacerbate side effects.

Dosage Concerns: Determining the optimal dosage for SAM, especially when combined with other medications, remains a challenge. Overdose or prolonged usage could result in unexpected side effects, while suboptimal doses might not provide the desired therapeutic benefits.

Long-term Effects: The long-term impacts of using SAM, particularly in conjunction with other medications, are yet to be fully understood. Potential side effects or complications arising from prolonged treatment need further investigation.

Generalizability: This study focuses on a combination approach of prenatal treatment and postnatal medications. The results and conclusions might not be generalizable to all PCOS patients, especially those not subjected to prenatal interventions.

Data Analysis: Our data analysis, while comprehensive, could still benefit from a larger sample size and a more diverse patient cohort to better represent the broader PCOS population.

4.3. Implication

After treatment with SAM, Changes in the expression of certain genes associated with PCOS, it indicates, SAM may have some potential therapeutic effects on PCOS, first, SAM post-treatment, Mice with PCOS recovered ovulation, Abnormal luteinins and testosterone hormone levels also normalized, Islet hyperplasia was also returned to normal after SAM administration, Genes for visceral fat and the hypothalamus, Inflammation and immune responses involved in the genes, Genes for ovarian function and insulin transport, Regulated hypothalamic gonad genes and other genes were all differentiated in their expression levels after SAM treatment, From mouse studies may speculate on the possible effects of SAM therapy application in humans.

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