Review

Therapeutic Potentials of Low-Dose Tacrolimus for Aberrant Endometrial Features in Polycystic Ovary Syndrome

Ahmad J. H. Albaghdadi [ID] and Frederick W. K. Kan *

Department of Biomedical and Molecular Sciences, Faculty of Health Sciences, Queen's University, Kingston, ON K7L 3N6, Canada; a.albaghdadi@queensu.ca
* Correspondence: kanfwk@queensu.ca; Tel.: +1-613-533-2863

Abstract: Polycystic ovary syndrome (PCOS) is a major anovulatory infertility affecting a great proportion of women of childbearing age and is associated with obesity, insulin resistance and chronic inflammation. Poor endometrial receptivity and recurrent implantation failure are major hurdles to the establishment of pregnancy in women with PCOS. The accumulating body of evidence obtained from experimental and clinical studies suggests a link between inherent adaptive and innate immune irregularities and aberrant endometrial features in PCOS. The use of conventional therapeutic interventions such as lifestyle modification, metformin and ovarian stimulation has achieved limited clinical success in restoring ovulation and endometrial receptivity in women with PCOS. Unlike other immunosuppressive drugs prescribed in the clinical management of autoimmune and inflammatory disorders that may have deleterious effects on fertility and fetal development, preclinical studies in mice and in women without PCOS but with repeated implantation failure revealed potential therapeutic benefits for the use of low-dose tacrolimus in treating female infertility. Improved systemic and ovarian immune functions, endometrial progesterone receptor and coreceptor expressions and uterine vascular adaptation to pregnancy were among features of enhanced progesterone-receptor sensitivity in the low-dose tacrolimus-treated mouse model of the disease. In this review, we have compiled available experimental and clinical data in literature on endometrial progesterone resistance and current therapeutic options, as well as mechanisms of actions and reported outcomes relevant to the potential therapeutic benefits for the use of low-dose tacrolimus in treating PCOS-associated female infertility.

Keywords: polycystic ovary syndrome (PCOS); endometrial progesterone resistance; tacrolimus; immunosuppression; chronic inflammation

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous endocrine disorder and a major cause of anovulatory infertility affecting 6–10% of women of reproductive age [1–3]. Human and experimental studies confirm that, in its typical form, this multisystemic hormonal disorder is characterized by oligo- and/or anovulation, as well as hyperandrogenism, and is often associated with obesity, insulin resistance, dyslipidemia, immune disturbances and increased risk of cardiovascular events [4]. After excluding other ovarian and endocrine disorders simulating PCOS, the diagnosis of the disease is exclusively dependent on two of three features: irregular menstrual cycles with oligo- or anovulation, and polycystic ovarian morphology (PCOM) together with clinical and/or biochemical evidence of hyperandrogenemia [5,6]. Recommendations from the international evidence-based guideline for the assessment and management of PCOS (i.e., the Rotterdam diagnostic criteria) characterized four phenotypes of the syndrome: (1) Type A (classic) presents with irregular cycles with hyperandrogenism (HA) and polycystic ovarian morphology (PCOM), (2) Type B (classic) presents with HA and irregular cycles, (3) Type C (ovulatory, nonclassical) presents with HA and PCOM, and finally (4) Type D (normo-androgenic)

presents with irregular cycles and PCOM [7] (Table 1). Beside hormonal disturbances, particularly hyperandrogenemia and associated chronic anovulation, low progesterone (P4) levels and ensuing oligo-menorrhea further leading to endometrial dysfunctions, loss of endometrial plasticity is believed to be among contributing factors in the development of the reported cyclical irregularities in PCOS [8]. It is generally held that combinations of primary (genetic and cellular) endometrial abnormalities manifesting during prenatal development, together with postnatal endometrial defects secondary to hormonal, metabolic, inflammatory and immune malfunctions, result in these PCOS-associated endometrial dysfunctional phenotypes [9,10]. Indeed, evidence of primary endometrial defects in women with PCOS is demonstrated in the existence of inherent endometrial aberrancies in both ovulatory and anovulatory phenotypes affecting several biological pathways and the anomalous expressions of proteins involved in endometrial receptivity, such as those related to cell adhesion and the cytoskeleton network, transcriptional regulation, DNA repair, apoptosis and cell cycle regulation, cellular transport and signaling and mitochondrial metabolism [11–15]. Importantly, evidence of perturbed endometrial immune responses and poor endometrial receptivity has also been reported in ovulatory and anovulatory women with PCOS [10,11,16,17].

Table 1. Classification of polycystic ovary phenotypes (modified from references [7,18]).

Parameter	Phenotype A	Phenotype B	Phenotype C	Phenotype D
HA	+	+	+	−
OD	+	+	−	+
PCOM	+	−	+	+

Abbreviations: HA: hyperandrogenism, OD: ovulatory dysfunction, PCOM: polycystic ovarian morphology.

2. Evidence of Endometrial Immune Irregularities in PCOS

Despite a plethora of emerging data and myriad suggested hypotheses on the immunological abnormalities associated with PCOS, the exact mechanism(s) underlying the immune etiology of this endocrine metabolic disorder and its links to disturbed endometrial functions remain moot. To date, the general consensus is that the onset of PCOS is marked by low-grade inflammation characterized by elevated serum levels of CRP, IL1, IL6 and products of activated monocytes (e.g., TNFα, IFNγ, MCP1, MCP5 and MIP) as well as molecules originated from leukocyte–endothelium interactions (e.g., VCAM-1, ICAM-1) [19–21]. Autoantibodies have also been detected in women with PCOS [22,23], and a causal association between chronic low-grade inflammation and the age- and adiposity-dependent development of endocrine, metabolic and cardiovascular adversities in PCOS has been reported [24,25]. At the endometrial level, impaired progesterone-mediated decidualization of endometrial stromal fibroblasts was associated with aberrant pro-inflammatory gene-expression profiles in the form of increased expression of IL8, monocyte chemo-attractant proteins MCP1 and MCP3 (CCL2 and CCL7, respectively), RANTES (CCL5) and granulocyte-macrophage colony-stimulating factor (GM-CSF) [17]. These have been reported among several endometrial cell populations in the proliferative phase endometrium of anovulatory women with PCOS [10,17]. Moreover, proteomic studies by Rashid et al. (2020) unraveled evidence of aberrant differential expression of proteins involved in inflammation, humoral immune responses, immunomodulation and immune-checkpoint mechanisms in women with PCOS with subdued fertility and dampened endometrial receptivity [11]. These findings are supported by the significant relationship between programmed cell death-1 (PD-1) and programmed cell death-1-ligand I (PD-L1) polymorphisms and PCOS, which was reported by Han et al. (2021) [16]. Altogether, the importance of the findings by Rashid et al. (2020) and Han et al. (2021) stems also from the critical role of the PD-1/PD-L1 immune-checkpoint pathway in modulating the cytotoxic activity of a subset of decidual CD8+ T cells, namely the PD1/NKG2D double positive CD8+ regulatory T cells (Tregs), which contribute to maternal immunotolerance at

the maternofetal interface [26]. Another important subset of endometrial Tregs involved in modulating local immune milieu during implantation are the CD4+CD25+ T cells [27]. The quintessential role of these fetal-specific Tregs in suppressing the allogeneic response against the implanting embryo and supporting early gestation is well demonstrated in previous animal studies [28–30]. Whereas adaptive transfer of exogenous Tregs in the peri-implantation period in depleted mice prevented fetal loss [29], targeted depletion of these T cells during implantation terminates pregnancy [28]. One major mechanism for the differentiation of these Tregs from their naïve CD4+ status is the activation and expression of the fate-determining transcriptional factor FoxP3, which are believed to be achieved under the influence of the modulatory cytokines TGF-β, IL2 and IL15 [27,31]. This critical differentiation of the CD4+ T cells into functioning FoxP3+ CD4+CD25+ Tregs might be defective in PCOS [32]. A study by Krishna et al. (2015) reported that the inability of the CD4+CD25+ T cells, extracted from the entire peripheral blood mononuclear cell population, to expand during the follicular phase is most likely a consequence of an inherent hyporesponsiveness to IL2 signaling and compromised STAT5 activation in women with PCOS [32]. STAT5 is necessary for Treg development, as it binds to the FoxP3 promoter and regulates FoxP3 expression [33]. Preclinical studies in mice have also revealed that the declining thymic ability of the estrogen-primed female mouse to produce CD4+CD25+ regulatory T cells culminated in anovulation and ovarian-cyst development [34]. Moreover, among features of perturbed Tregs functions in PCOS is the low-level expression of the leukemia inhibitory factor (LIF) in the endometria of women with PCOS [35]. LIF is a Treg cytokine that influences fate determination in these cells [36,37]. More importantly, animal studies and clinical data support a critical role for LIF in preparing the endometrium for embryo implantation and postimplantation embryonic development [38,39]. Therefore, the crucial role of FoxP3+ Tregs in endometrial receptivity and embryo implantation [30,40,41] dictates the need for alternative therapeutic approaches to support endometrial FoxP3 activation and the periconceptional expansion of the CD4+CD25+ Tregs in infertile women [27], especially those with PCOS.

3. Evidence of Endometrial Progesterone Resistance in PCOS

A resistance to the hormonal actions of progesterone (P4), also referred to as P4 resistivity, has been described in women with infertility, including those with PCOS [42–44]. Human studies established that PCOS endometrium is inherently different with respect to its response to progesterone [44,45]. PCOS patients exhibit poor endometrial receptivity and reduced effectiveness and exposure to progesterone [46]. Molecular mechanisms associated with the latter are not well understood. However, quantitative and qualitative alterations in the endometrial epithelial progesterone receptor (PGR) have been described in the secretory endometrium of women with PCOS [46–48]. Quezada et al. (2006) confirmed that higher levels of expression of the endometrial epithelial PGR messenger RNA (mRNA) and protein are associated with an aberrant higher expression of the androgen receptor associated protein 70 (ARA70) and low level of the adhesion molecule beta-3 integrin in the midsecretory endometrium of women with PCOS [46]. Aberrancies in the endometrial epithelial expressions of PGR-A and PGR-B have also detected in the proliferative phase of chronically anovulatory obese women with PCOS [47]. Studies by Paulson et al. (2017) revealed that the aberrant overexpression of the endometrial epithelial and stromal PGR-B and the downregulation of the PGR-A in the proliferative endometrium of obese women with PCOS likely impacted proliferation of the secretory-phase endometrium [47]. Features of defective endometrial progesterone actions have also been confirmed by Hu et al. (2018) [48], suggesting that the aberrant overexpression of PGR-B in the cytoplasm rather than the nuclei of the endometrial epithelial and stromal cells is associated with the development of PCOS in women [48]. The culprit of the abovementioned irregularities of the endometrial PGR expression and localization is believed to be, in part, due to chronic anovulation and the elevated levels of androgens and their receptors, estrogen receptor alpha (ERα) and steroid receptor co-activators in the PCOS endometrium [48,49]. How-

ever, the plausible mechanisms involved in this progesterone resistance may reside in the progesterone receptor (PGR) itself. This was first reported by Chrousos et al. (1986) [50] and is supported by the work of Igarashi et al. (2005) [51] establishing that alteration in the pattern of endometrial PGR expression leads to endometrial P4 resistivity [51]. According to their cellular locations and mechanisms of actions, progesterone receptors are either located at the cell membrane, also referred to as PGRM with its two identified component isoforms: PGRMC1 and PGRMC2 conveying the rapid non-genomic actions of P4, and the cytosolic/nuclear PGR (PGR-A and PGR-B) mediating the genomic actions of the hormone [52]. Aberrancies in the expressions of PGR (A and B), PGRMC1 and PGRMC2 in the endometria of anovulatory women with PCOS have been reported [47]. Compared to the control group of regularly cycling women, Paulson et al. (2017) [47] observed persistent higher expressions of PGR (A and B), PGRMC1 and PGRMC2 in the stroma and of PGRMC1 in the luminal epithelium on cycle days 21–23 in obese anovulatory women with PCOS [47]. These findings suggest inherent defects in the cytosolic and nuclear PGR functions and expression in women with PCOS. The cytosolic/nuclear PGR is a heterodimer of the naïve progesterone receptor protein in dynamic interactions with its chaperones HSP90, HSP70 and p23, and its cochaperone immunophilins FKBP51 and FKBP52 [53–56]. Ligand binding causes the release of multiple HSP-subunit complexes, allowing the PGR to undergo certain conformational changes assisted by the enzymatic action of the FKBPs, which permits the receptor dimer to interact with specific progesterone response elements localized at the regulatory regions of its target genes [57,58]. Studies showed that alterations in the expression or function of PGR coactivators, chaperones, and cochaperones that are bound to the mature form of the cytosolic PGR before activation and nuclear translocation have been implicated in the development of P4 resistivity [59–61]. Although few investigations have been conducted on the impact of FKBPs on PCOS, the general consensus is that lack of cochaperone FKBP52 or the overexpression of the cochaperone FKBP51 causes PGR signaling irregularities and endometrial progesterone resistance in experimental murine models [61,62]. Furthermore, among the critical events involved in the generation of a receptive endometrium are the dynamic interactions between endometrial PGR and other transcriptional factors such as the Forkhead box protein O1 (FoxO1) [63,64]. Human and mouse studies confirmed the existence of a vital molecular switch mechanism represented by the reciprocal relationship between endometrial epithelial PGR and FoxO1 expression and activation during the window of receptivity [63,64]. FoxO1 protein is a key mediator of insulin action on gene expressions [65] and is a negative regulator of cell survival that inhibits cell proliferation and promotes cell apoptosis and cycle arrest [66]. Aberrant overexpression and activation of FoxO1 have been reported in women with PCOS [67,68]. Studies by the Vega group suggested a mechanistic link between a persistent high level expression of the phosphorylated FoxO1 (p-FoxO1Ser319) in the endometrial epithelial compartment and disturbed endometrial homeostasis, steroid bioavailability and failed uterine receptivity in obese and hyperinsulinemic women with PCOS [46,68]. Additionally, the aberrantly upregulated FoxO1 was also found to mediate the production of proinflammatory cytokines that alter the PGR expression, such as IL1β, IL6 and TNF-α, in women with PCOS [67]. While these reports may suggest a role of abnormal FoxO1 signaling in the development of restricted progesterone-dependent uterine receptivity and decidualization in PCOS, further molecular studies into the mechanistic association between the endometrial PGR and other transcriptional mediators of endometrial receptivity, particularly the Indian hedgehog (IHH) signaling pathway, are warranted. Notably, the fact that the persistence of the abovementioned endometrial irregularities despite adequate metabolic control, lifestyle interventions and the restoration of regular ovulatory cycles [47,69] underpins the need to further examine the link between chronic inflammation and related immunological abnormalities in the development of P4 resistivity in PCOS.

4. Current Therapeutic Options for PCOS

Current consensus guidelines and clinical accord on the management of anovulatory infertility in women with PCOS focus largely on improving ovulatory function and managing oligo- or anovulation-related subfertility [5,70]. Therefore, irrespective of their FDA-approval status, current therapeutic interventions for PCOS include lifestyle modification and weight management, as well as drugs that induce ovulation, such as clomiphene citrate (CC) and aromatase inhibitors (such as letrozole and/or anastrozole) [71–74]. Additionally, combined oral contraceptive pills (OCPs) have been used to improve hyperandrogenism in women with PCOS [75], and for its demonstrated clinical and metabolic benefits improving ovulation and menstrual frequency in anovulatory PCOS patients, the insulin-sensitizing agent metformin has also been prescribed [76]. Weight loss is considered an initial treatment strategy for reproductive disorders in overweight and obese women with PCOS [25]. Evidence showed that active lifestyle management, weight loss and physical activity help menstrual disturbances and can shorten the time to conception and reduce adverse obstetric risks [77]. However, the current management of the irregular menstrual cycles to protect the endometrium against development of hyperplasia entails the use of OCPs [78]. The flip side of the use of OCPs in PCOS not only resides in the increased risk of inflammatory and coagulatory disorders and cardiovascular-disease development [79,80], but also in the lack of sufficient data from randomized clinical trials comparing progestins alone versus combined estrogens and progestins in the treatment of irregular vaginal bleeding due to anovulation. This has cautioned the need for alternative therapeutic approaches [81].

Among insulin-sensitizing agents primarily used to treat insulin resistance with demonstrated clinical and metabolic benefits in the management of PCOS in women is metformin [82,83]. Although metformin is not approved by the US Food and Drug Administration (FDA) for the treatment of infertility in women with PCOS [84], its use in PCOS is largely derived from case-control studies with occasional conflicting results [85–87]. A prospective evaluation of the safety of metformin administration during pregnancy in 98 women with PCOS done by De-Leo and colleagues (2011) [86] found a significant reduction in pregnancy complications such as gestational diabetes mellitus (GDM) and gestational hypertension [86]. On the other hand, data obtained from a randomized double-blind placebo-controlled trial conducted by Vanky and associates (2010) [85] revealed no difference in the primary outcome, which was a composite of preeclampsia, spontaneous abortion, GDM and preterm delivery among 257 women with PCOS receiving metformin (500–1000 mg twice daily) from the first trimester to delivery [85]. Nonetheless, besides its systemic and ovarian actions in reducing peripheral insulin resistance, regulating hepatic glucose release and inhibiting androgen production in the ovaries, experimental and clinical data showed that metformin may have a direct modulatory effect on the endometrium [83,88,89]. Experimental studies revealed that metformin could ameliorate uterine receptivity defects and partially improve implantation rates, and had significant immunosuppressive properties when used at a high dosage in the treatment of PCOS-associated adverse pregnancy outcomes in murine models of the disease [90,91]. In doing so, the molecular mode of action of metformin on the endometrium seems to be diverse. A study by Zhai et al. (2019) [92] suggested that metformin may improve endometrial receptivity through increasing the expression of the homeobox A10 (HOXA10) and integrin beta-3 (ITGB3) via the downregulation of miR-1910-3p and miR-491-3p in the endometrium of women with PCOS [92]. These findings are supported by recent data from Zhang et al. (2020) [93] on the modulatory effect of metformin on the activation of the AMPK signaling pathway in the endometrium of diabetic pregnant mice in improving implantation rates [93]. Moreover, in a study reported by Xiong et al. (2019) [94], metformin was found to alleviate estradiol and progesterone-induced decidualization of human endometrial stromal cells by modulating the secretion of multiple cytokines, inhibiting expression of matrix metalloproteinase-2 and -9, activating MAPK/ERK/p38 signaling and reducing PGR expression [94]. Metformin can also normalize androgen-receptor-mediated transcriptions, thereby restoring endometrial epithelial–stromal crosstalk, and has significant

antagonizing actions in reversing the androgen-induced alterations in the insulin receptor substrate and GLUT4 expressions in endometrial glandular epithelial cells [95,96]. However, notwithstanding the additional health benefits for the use of metformin in reducing serum atherogenic biomarkers, such as the advanced glycation end products and C-reactive protein [79], the role of metformin in treating PCOS is narrowing [97]. Metformin may improve the menstrual cycle within 1–3 months; nonetheless, multiple reports from systemic reviews and randomized clinical trials indicate that metformin may not improve implantation and/or live birth rates or reduce miscarriage in women with PCOS [98–100]. More importantly, the use of metformin during pregnancy did not reduce maternal weight gain or avert gestational diabetes mellitus when initiated during pregnancy in at-risk women [101,102], nor did it reduce the relatively high obstetrical risk associated with increased rates of cesarean sections and the delivery of babies that were large for their gestational age and macrosomic babies (i.e., >4 or 4.5 kg at birth) [101–103].

Unlike metformin, the use of ovulation-induction agents such as the aromatase inhibitor letrozole has gained popularity [104–106]. Letrozole is effective for ovulation induction, and new data show improved live birth rates by skipping a progestin withdrawal bleed and proceeding directly with a dose escalation of letrozole in clomiphene citrate (CC)-resistant women with PCOS [77,106]. However, unlike CC, letrozole is categorized as pregnancy class D drug and is not FDA-approved for treating infertility and inducing ovulation [74]. Nonetheless, experimental and clinical data have indicated better effects of letrozole compared to CC on endometrial thickness and the expression of endometrial receptivity markers, and those associated with embryo implantation and placental development such as the Wnt-beta-catenin pathway critical for embryo implantation [107,108]. All in all, the safety and efficacy of letrozole or clomiphene citrate in achieving live birth in infertile women with PCOS will need to be further evaluated.

Other therapeutic modality in the management of PCOS-associated female infertility is the use of gonadotropins [77,109]. Ovulation induction with follicle-stimulating hormone (FSH) is currently reserved as a second-line treatment for anovulatory women with PCOS who fail to respond to CC or letrozole [77]. Irrespective of the source and clinical formulation, clinical data and meta-analyses indicated little or no difference in live birth rates, multiple pregnancy rates, clinical pregnancy rates or miscarriage rates between urinary-derived gonadotropins and recombinant FSH in women with PCOS [109]. Moreover, data obtained from large multicenter randomized clinical trials, such as the Assessment of Multiple Intrauterine Gestations from Ovarian Stimulation (AMIGOS) trial involving a cohort of 900 couples with unexplained infertility that included women with PCOS, supported a higher efficacy of gonadotropins compared to CC or letrozole for ovulation induction [110]. More important is the downside for the use of gonadotropins, which is higher rates of multiple pregnancies compared to CC or letrozole, which is hard to predict using clinical or biochemical markers of androgenic activity such as the serum concentrations of androgens [110,111]. A list of current pharmacological agents commonly used for treating anovulatory infertility in women with PCOS is summarized in Table 2.

Table 2. Mode of action and fertility-related side effects of current pharmacological agents * commonly used in anovulatory women with PCOS.

Medication	Mode of Action	Fertility-Related Side Effects
Clomiphene Citrate	- Induces ovarian follicular development [112]	- Due to its antiestrogenic effect, endometrial proliferation may be hampered [112,113] - May change cervical mucus characteristics with a consequent reduction in sperm penetration [112,113] - May impair endometrial receptivity [114] - May worsen subendometrial/endometrial vascularization as detected by power Doppler [114] - Its use is associated with an increased cardiac anomaly rate [115,116] - Resistance to CC is fairly common in women with PCOS (~ 15% of women with PCOS may not respond to the maximum dose of CC and are considered resistant to this medication) [78]
Gonadotropins (recombinant follicle-stimulating hormone (rFSH) or human menopausal gonadotropin (HMG))	- Stimulates endogenous peak of luteinizing hormone for oocyte maturation and ovulation triggering [117]	- Rate of clinical complications such as ovarian hyperstimulation ranges between 6.67 and 17.78% [118]; nonetheless, hCG therapy for PCOS-associated infertility is considered of high clinical curative value [118]
Letrozole	- Induces ovarian follicular growth and development and supports postovulatory corpus luteum without having a premature luteinizing effect on the developing follicles [119] - Supports endometrial proliferation through the stimulation of the Wnt/β-catenin pathway [107] - May improve endometrial receptivity and spiral artery resistivity indices [72,107,120] - Inhibits androgen-induced stimulation of E2 and aromatase p450 in endometrial cells [121]	- May result in elevated serum level of FSH [119] - May induce ovarian cyst formation [119] (not reported in CC-resistant women with PCOS [122]) - Can induce PCOS-like ovarian phenotype in experimental animals [123]
Metformin	- Improves glucose intolerance [87,88] - Modulates uterine/endometrial ER and PGR expressional irregularities [89] - Suppresses AR expression and normalizes the AR-mediated gene transcription [91,124] - May improve endometrial receptivity defects and improves uterine vascularity and subendometrial blood flow [125]	- Does not prevent the development of GDM [102] - May not improve live birth rate [126] - May not reduce the risk of PCOS-associated adverse pregnancy outcomes such as spontaneous abortion, gestational hypertension, preeclampsia and placental abruption [85] - Causes increased birth weight [103]

Abbreviations: CC: clomiphene citrate, FSH: follicle-stimulating hormone, GDM: gestational diabetes mellitus, ER: estrogen receptor, PGR: progesterone receptor, E2: estradiol, hCG: human chorionic gonadotropin. * Irrespective of current FDA-approval status.

5. Mode of Action of Tacrolimus and Its Potentials to Mitigate Progesterone Resistance and Associated Menstrual and Endometrial Abnormalities in PCOS

Tacrolimus (FK506) is a lipophilic immunosuppressant with a 23-membered ring macrolide macrolactam structure [127] that is firmly established in the clinical routine of immunosuppression. Recently, tacrolimus has been successfully prescribed in the preclinical management of PCOS-related female infertility in an obese murine model of PCOS [128] and in treating women without PCOS but with recurrent implantation failure (RIF)/recurrent pregnancy loss (RPL) with elevated systemic Th1 (CD4+ IFNγ+):Th2 (CD4+IL4+) cell ratios [129]. The first identified mode of action of tacrolimus is the in-

hibition of the T-cell receptor (TCR)-mediated Ca^{++}-dependent activation of the nuclear transcriptional factors NFκB and the nuclear factor of activated T cells (NFAT) [130]. This action is primarily contingent upon the binding of tacrolimus (FK506) to the FK506-binding protein 12 (FKBP12) and the subsequent inhibition of the phosphatase activity of the Ca^{++}-dependent serine/threonine (Ser/Thr) protein phosphatase calcineurin, thereby suppressing the dephosphorylation and nuclear translocation of NFκB and NFAT in a dose-dependent manner [131–135]. The primary outcome of this tacrolimus-suppressed TCR signaling is the restricted release of INFγ and IL2, as well as TNFα and GM-CSF, in activated peripheral blood monocytes and plasmacytoid dendritic cells in a dose-dependent manner [136,137]. Importantly, binding of tacrolimus to other members of the tetratricopeptide repeat (TPR) domain-containing FKBPs, particularly FKBP52, has been identified and has proven to influence varieties of physiological functions, particularly those mediated by the glucocorticoid receptors (GR) [138,139]. It has been shown that this binding of tacrolimus to the GR/cochaperone complex could increase the GR receptor transactivity and hormone-binding affinity [139,140]. These TPR cochaperones exhibit peptidylprolyl cis-trans isomerase (PPlase) activity, which, upon activation through the binding of tacrolimus, allows for the release of the mature form of the glucocorticoid receptors from the complex and their subsequent nuclear translocation for the mediation of their genomic actions [141,142]. In fact, the critical role of FKBP52 in the nuclear translocation of steroid receptors has been documented in a variety of cellular contexts [58,143]. The cytoplasmic fraction of FKBP52 is localized to microtubules and serves as an adaptor between dynein, which is attached to the PPlase domain of the protein and the TPR domain-bound GR/HSP90 complex of the steroid receptor [58,144]. Davis et al. (2002) [145] reported a switch mechanism in which the hormone causes exchange of the inhibitory cochaperone FKBP51 for the stimulatory FKBP52 in the GR complex [145]. This exchange has also been shown to influence the corecruitment of the dynein motor protein and movement of the mature GR complex to the nucleus for downstream genomic signaling [145].

Moreover, tacrolimus can modulate the transcriptional activities of the PGR through mechanisms involving the activation of the protein inhibitor of activated STAT-y (PIASy) [146]. PIASy is a member of the PIAS family E3-type small ubiquitin-like modifiers (SUMO) capable of transcriptionally repressing the PGR receptors directly through SUMOylation or indirectly via binding to the steroid receptor DNA-binding domain [147]. We have previously shown that PIASy is aberrantly downregulated and that it has low affinity to be recruited to the nuclear PGR in the peri-implantation uterus of an obese mouse model of PCOS [146]. We have also demonstrated that the use of low-dose tacrolimus rescued the endometrial expression of PIASy and restored its binding capacity to interact with and regulate the transcriptional activity of the nuclear PGR in these uteri [146]. Previous studies showed that the SUMOylation of the PGR, particularly PGR-B, by members of the E3-ligaeses destabilizes its nuclear retention, thereby repressing its transactivation and downstream genomic signaling [148]. Additionally, this PIASy-mediated alterations of PGR phosphorylation rate and the subsequent reduction in its nuclear shuffling and retention efficiency are also mediated by the concerted actions of the histone deacetylases, which bind to active DNA-binding sites, such as those abundantly located within the hinge region of the PGR-A [149,150]. This PIASy-controlled downregulation of PGR-A is histone deacetylases-1-mediated in ex vivo cultured human primary myometrial cells [151]. This is important to our understanding of the mode of action of tacrolimus in restoring endometrial receptivity in PCOS. Both PGR-A and PGR-B were aberrantly expressed during the window of receptivity in the uteri of obese mice with PCOS [146]. The prepregnancy administration of low-dose tacrolimus restored normalized expressions of PGR-A and PGR-B and their interactions with PIASy at the implantation window conducive to gestational success in these mice [146]. This was associated with increased implantation and live birth rates among treated mice [90,146]. Therefore, we believe that through these PGR-regulatory effects, the use of low-dose tacrolimus suppresses heightened endometrial progesterone

resistance, promotes endometrial receptivity and aids in the prevention of implantation failure in a murine model of PCOS [146].

6. Tacrolimus and Its Potentials to Prevent Dysregulated Treg Response in PCOS

The interplay between the ovarian follicular cells and the effector arm of the follicular immune system, of which the regulatory T cells (Tregs) are critical effectors [152], is indispensable for the control of ovulation [153]. Residents as well as infiltrating lymphocytes contribute substantially to the cyclic-tissue remodeling of the ovary due to their ability to secrete various inflammatory and immunomodulating molecules [152]. As described earlier, among the immunosuppressive regulatory T cells are those defined by their signature expression of the cell-surface molecules CD4, CD25 and the transcriptional factor FoxP3 [154–156]. Notwithstanding the limited sample size reported in the human studies, an accumulating body of evidence from animal and human data implicates evident immunological deficits among circulating and ovarian resident Tregs in the pathogenesis of PCOS [32,128,157]. Of the latter are the reduced expression of FoxP3 and decreased expansion of CD4+CD25+CD127low Tregs due to inherent aberrancies in Interleukin 2 (IL2) signaling in women with PCOS [32]. This is valuable to our understanding of the usefulness of low-dose tacrolimus (i.e., ≤10 ng/mL) in mitigating these inherit immunological deficits in PCOS. Firstly, tacrolimus has the potential to bidirectionally regulate the transcriptional activities of FoxP3 in a variety of cellular contexts [158]. Shen et al. (2011) [158] confirmed that this tacrolimus-mediated action on FoxP3 is dose-dependent, and a low concentration of 10 ng/mL tacrolimus resulted in higher nuclear shuffling of the nuclear factor of activated T cells (NFAT) [158]. Members of the NFAT family expressed by the immune cells, including NFAT1, NFAT2 and NFAT4, directly bind to the FoxP3 enhancer and/or cooperate with Smad3 to activate FoxP3 transcription, thereby regulating the production of cytokines by T cells [159]. These diversified actions of NFAT on FoxP3 transcription are contingent upon their calcineurin-mediated dephosphorylation, subsequent nuclear translocation and unmasking of at least six of their T-cell-specific FoxP3 nuclear localization sequences, which positively regulate the transactivation of FoxP3 gene after triggering of the T-cell receptor (TCR) [160,161]. While translating the actions of low-dose tacrolimus on FoxP3 activation in PCOS awaits further investigations, we believe that, at least in part, through this plausible stimulatory effect on FoxP3, low-dose tacrolimus is capable of inducing the periconceptional expansion of the CD4+CD25+CD127low Tregs in a murine model of PCOS [128]. Second, tacrolimus has the potential to moderate the severity of the compromised cross-talks between resident white blood cells, such as macrophages with systemic T helper cells Th1 (CD4+ IFNγ+), Th2 (CD4+IL4+) and Th17 (CD4+IL17A+) [162,163] critically involved in the process of expulsing the oocyte from the antral/Graafian follicles and maintaining a fetal-protective decidual immune milieu during gestation [30,152]. These modulatory effects of low-dose tacrolimus on the expression and activation profiles of the Th1 (CD4+ IFNγ+)/Th2 (CD4+IL4+) and Th17 (CD4+IL17A+) Tregs have indeed been proven effective in the clinical management of women without PCOS but with recurrent implantation failure [129] and in a murine model of PCOS [128], respectively.

7. Conclusions

Provision of the best care for women with PCOS requires thorough understanding of the underlying immune and molecular mechanisms associated with poor ovarian functions, heightened progesterone resistance and declining endometrial receptivity. The persistence of clinical, immunological and histopathological features of endometrial malfunction, despite the use of the most effective ovarian stimulation regimen, dictate the need for further investigations into the fundamental molecular and immunological mechanisms of ailing endometrial health in women with PCOS. Evidence presented in this review suggests that immunomodulation with low-dose tacrolimus may mitigate the severity of PCOS-associated female infertility. The efficacy of tacrolimus to promote endometrial receptivity may reside in its intrinsic ability to regulate the endometrial progesterone receptor signaling

while suppressing systemic immune aberrancies and associated endometrial immune irregularities in PCOS. Lastly, while the present data from experimental and human studies point to the relative perinatal safety for the use of low-dose tacrolimus in treating female infertility [90,164], further studies are needed to establish the best-fit tacrolimus-based monotherapeutic interventions in the management of PCOS-associated female infertility.

Author Contributions: A.J.H.A. prepared the original draft. A.J.H.A. and F.W.K.K. contributed equally to the conceptualization, review, editing, and approval of the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This review article did not report any previously unpublished research data. All information contained in this review article can be retrieved online at https://www.ncbi.nlm.nih.gov/ (accessed on 2 February 2021).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mohammad, M.B.; Seghinsara, A.M. Polycystic Ovary Syndrome (PCOS), Diagnostic Criteria, and AMH. *Asian Pac. J. Cancer Prev.* **2017**, *18*, 17–21. [CrossRef]
2. Zangeneh, F.Z.; Naghizadeh, M.M.; Masoumi, M. Polycystic ovary syndrome and circulating inflammatory markers. *Int. J. Reprod. Biomed.* **2017**, *15*, 375–382. [CrossRef]
3. Wang, L.; Qi, H.; Baker, P.N.; Zhen, Q.; Zeng, Q.; Shi, R.; Tong, C.; Ge, Q. Altered Circulating Inflammatory Cytokines Are Associated with Anovulatory Polycystic Ovary Syndrome (PCOS) Women Resistant to Clomiphene Citrate Treatment. *Med. Sci. Monit.* **2017**, *23*, 1083–1089. [CrossRef] [PubMed]
4. Boots, C.E.; Jungheim, E.S. Inflammation and Human Ovarian Follicular Dynamics. *Semin. Reprod. Med.* **2015**, *33*, 270–275. [CrossRef] [PubMed]
5. Legro, R.S.; Arslanian, S.A.; Ehrmann, D.A.; Hoeger, K.M.; Murad, M.H.; Pasquali, R.; Welt, C.K.; Endocrine, S. Diagnosis and treatment of polycystic ovary syndrome: An Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 4565–4592. [CrossRef] [PubMed]
6. Amsterdam, E.A. Consensus on women's health aspects of polycystic ovary syndrome (PCOS). *Hum. Reprod.* **2012**, *27*, 14–24. [CrossRef]
7. Teede, H.J.; Misso, M.L.; Costello, M.F.; Dokras, A.; Laven, J.; Moran, L.; Piltonen, T.; Norman, R.J. International PCOS Network. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum. Reprod.* **2018**, *33*, 1602–1618. [CrossRef]
8. Palomba, S.; Piltonen, T.T.; Giudice, L.C. Endometrial function in women with polycystic ovary syndrome: A comprehensive review. *Hum. Reprod. Update* **2020**. [CrossRef]
9. Yan, L.; Wang, A.; Chen, L.; Shang, W.; Li, M.; Zhao, Y. Expression of apoptosis-related genes in the endometrium of polycystic ovary syndrome patients during the window of implantation. *Gene* **2012**, *506*, 350–354. [CrossRef]
10. Piltonen, T.T.; Chen, J.; Erikson, D.W.; Spitzer, T.L.; Barragan, F.; Rabban, J.T.; Huddleston, H.; Irwin, J.C.; Giudice, L.C. Mesenchymal stem/progenitors and other endometrial cell types from women with polycystic ovary syndrome (PCOS) display inflammatory and oncogenic potential. *J. Clin. Endocrinol. Metab.* **2013**, *98*, 3765–3775. [CrossRef]
11. Rashid, N.; Nigam, A.; Jain, S.K.; Naqvi, S.H.; Wajid, S. Proteomic sift through serum and endometrium profiles unraveled signature proteins associated with subdued fertility and dampened endometrial receptivity in women with polycystic ovary syndrome. *Cell Tissue Res.* **2020**, *380*, 593–614. [CrossRef]
12. Amjadi, F.; Mehdizadeh, M.; Ashrafi, M.; Nasrabadi, D.; Taleahmad, S.; Mirzaei, M.; Gupta, V.; Salekdeh, G.H.; Aflatoonian, R. Distinct changes in the proteome profile of endometrial tissues in polycystic ovary syndrome compared with healthy fertile women. *Reprod. Biomed. Online* **2018**, *37*, 184–200. [CrossRef]
13. Gonzalez, D.; Thackeray, H.; Lewis, P.D.; Mantani, A.; Brook, N.; Ahuja, K.; Margara, R.; Joels, L.; White, J.O.; Conlan, R.S. Loss of WT1 expression in the endometrium of infertile PCOS patients: A hyperandrogenic effect? *J. Clin. Endocrinol. Metab.* **2012**, *97*, 957–966. [CrossRef] [PubMed]
14. Paravati, R.; De Mello, N.; Onyido, E.K.; Francis, L.W.; Brusehafer, K.; Younas, K.; Spencer-Harty, S.; Conlan, R.S.; Gonzalez, D.; Margarit, L. Differential regulation of osteopontin and CD44 correlates with infertility status in PCOS patients. *J. Mol. Med.* **2020**, *98*, 1713–1725. [CrossRef]

15. Alikhani, M.; Amjadi, F.; Mirzaei, M.; Wu, Y.; Shekari, F.; Ashrafi, M.; Mehdizadeh, M.; McKay, M.; Taleahmad, S.; Aghajan-pour, S.; et al. Proteome analysis of endometrial tissue from patients with PCOS reveals proteins predicted to impact the disease. *Mol. Biol. Rep.* **2020**, *47*, 8763–8774. [CrossRef]

16. Han, R.; Gong, X.; Zhu, Y.; Liu, X.; Xia, Y.; Huang, Y.; Zhang, M.; Zhang, Y.; La, X.; Ding, J. Relationship of PD-1 (PDCD1) and PD-L1 (CD274) Single Nucleotide Polymorphisms with Polycystic Ovary Syndrome. *Biomed Res. Int.* **2021**, *2021*. [CrossRef]

17. Piltonen, T.T.; Chen, J.C.; Khatun, M.; Kangasniemi, M.; Liakka, A.; Spitzer, T.; Tran, N.; Huddleston, H.; Irwin, J.C.; Giudice, L.C. Endometrial stromal fibroblasts from women with polycystic ovary syndrome have impaired progesterone-mediated decidu-alization, aberrant cytokine profiles and promote enhanced immune cell migration in vitro. *Hum. Reprod.* **2015**, *30*, 1203–1215. [CrossRef] [PubMed]

18. Lizneva, D.; Suturina, L.; Walker, W.; Brakta, S.; Gavrilova-Jordan, L.; Azziz, R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil. Steril.* **2016**, *106*, 6–15. [CrossRef]

19. Seow, K.M.; Juan, C.C.; Wang, P.H.; Ho, L.T.; Hwang, J.L. Expression levels of vascular cell adhesion molecule-1 in young and nonobese women with polycystic ovary syndrome. *Gynecol. Obset. Investig.* **2012**, *73*, 236–241. [CrossRef] [PubMed]

20. Solano, M.E.; Sander, V.A.; Ho, H.; Motta, A.B.; Arck, P.C. Systemic inflammation, cellular influx and up-regulation of ovarian VCAM-1 expression in a mouse model of polycystic ovary syndrome (PCOS). *J. Reprod. Immunol.* **2011**, *92*, 33–44. [CrossRef]

21. Victor, V.M.; Rovira-Llopis, S.; Banuls, C.; Diaz-Morales, N.; Martinez de Maranon, A.; Rios-Navarro, C.; Alvarez, A.; Gomez, M.; Rocha, M.; Hernandez-Mijares, A. Insulin Resistance in PCOS Patients Enhances Oxidative Stress and Leukocyte Adhesion: Role of Myeloperoxidase. *PLoS ONE* **2016**, *11*, e0151960. [CrossRef]

22. Palacio, J.R.; Iborra, A.; Ulcova-Gallova, Z.; Badia, R.; Martinez, P. The presence of antibodies to oxidative modified proteins in serum from polycystic ovary syndrome patients. *Clin. Exp. Immunol.* **2006**, *144*, 217–222. [CrossRef] [PubMed]

23. Arduc, A.; Aycicek Dogan, B.; Bilmez, S.; Imga Nasiroglu, N.; Tuna, M.M.; Isik, S.; Berker, D.; Guler, S. High prevalence of Hashimoto's thyroiditis in patients with polycystic ovary syndrome: Does the imbalance between estradiol and progesterone play a role? *Endocr. Res.* **2015**, *40*, 204–210. [CrossRef] [PubMed]

24. Dumesic, D.A.; Oberfield, S.E.; Stener-Victorin, E.; Marshall, J.C.; Laven, J.S.; Legro, R.S. Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. *Endocr. Rev.* **2015**, *36*, 487–525. [CrossRef] [PubMed]

25. Orio, F.; Palomba, S. Reproductive endocrinology: New guidelines for the diagnosis and treatment of PCOS. *Nat. Rev. Endocrinol.* **2014**, *10*, 130–132. [CrossRef] [PubMed]

26. Meggyes, M.; Miko, E.; Szigeti, B.; Farkas, N.; Szereday, L. The importance of the PD-1/PD-L1 pathway at the maternal-fetal interface. *BMC Pregnancy Childbirth* **2019**, *19*, 74. [CrossRef]

27. Guerin, L.R.; Prins, J.R.; Robertson, S.A. Regulatory T-cells and immune tolerance in pregnancy: A new target for infertility treatment? *Hum. Reprod. Update* **2009**, *15*, 517–535. [CrossRef]

28. Aluvihare, V.R.; Kallikourdis, M.; Betz, A.G. Regulatory T cells mediate maternal tolerance to the fetus. *Nat. Immunol.* **2004**, *5*, 266–271. [CrossRef] [PubMed]

29. Zenclussen, A.C.; Gerlof, K.; Zenclussen, M.L.; Sollwedel, A.; Bertoja, A.Z.; Ritter, T.; Kotsch, K.; Leber, J.; Volk, H.D. Abnormal T-cell reactivity against paternal antigens in spontaneous abortion: Adoptive transfer of pregnancy-induced CD4+CD25+ T regulatory cells prevents fetal rejection in a murine abortion model. *Am. J. Pathol.* **2005**, *166*, 811–822. [CrossRef]

30. Robertson, S.A.; Moldenhauer, L.M. Immunological determinants of implantation success. *Int. J. Dev. Biol.* **2014**, *58*, 205–217. [CrossRef]

31. Burchill, M.A.; Yang, J.; Vang, K.B.; Farrar, M.A. Interleukin-2 receptor signaling in regulatory T cell development and homeostasis. *Immunol. Lett.* **2007**, *114*, 1–8. [CrossRef] [PubMed]

32. Krishna, M.B.; Joseph, A.; Subramaniam, A.G.; Gupta, A.; Pillai, S.M.; Laloraya, M. Reduced Tregs in peripheral blood of PCOS patients—A consequence of aberrant Il2 signaling. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 282–292. [CrossRef] [PubMed]

33. Burchill, M.A.; Yang, J.; Vogtenhuber, C.; Blazar, B.R.; Farrar, M.A. IL-2 receptor beta-dependent STAT5 activation is required for the development of Foxp3+ regulatory T cells. *J. Immunol.* **2007**, *178*, 280–290. [CrossRef] [PubMed]

34. Chapman, J.C.; Min, S.H.; Freeh, S.M.; Michael, S.D. The estrogen-injected female mouse: New insight into the etiology of PCOS. *Reprod. Biol. Endocrinol.* **2009**, *7*, 47. [CrossRef]

35. Kara, M.; Ozcan, S.S.; Aran, T.; Kara, O.; Yilmaz, N. Evaluation of Endometrial Receptivity by Measuring HOXA-10, HOXA-11, and Leukemia Inhibitory Factor Expression in Patients with Polycystic Ovary Syndrome. *Gynecol. Minim. Invasive Ther.* **2019**, *8*, 118–122. [CrossRef] [PubMed]

36. Metcalfe, S.M. LIF in the regulation of T-cell fate and as a potential therapeutic. *Genes Immun.* **2011**, *12*, 157–168. [CrossRef]

37. Janssens, K.; Van den Haute, C.; Baekelandt, V.; Lucas, S.; van Horssen, J.; Somers, V.; Van Wijmeersch, B.; Stinissen, P.; Hendriks, J.J.; Slaets, H.; et al. Leukemia inhibitory factor tips the immune balance towards regulatory T cells in multiple sclerosis. *Brain Behav. Immun.* **2015**, *45*, 180–188. [CrossRef]

38. Sharkey, A.M.; Dellow, K.; Blayney, M.; Macnamee, M.; Charnock-Jones, S.; Smith, S.K. Stage-specific expression of cytokine and receptor messenger ribonucleic acids in human preimplantation embryos. *Biol. Reprod.* **1995**, *53*, 974–981. [CrossRef]

39. Stewart, C.L.; Kaspar, P.; Brunet, L.J.; Bhatt, H.; Gadi, I.; Kontgen, F.; Abbondanzo, S.J. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* **1992**, *359*, 76–79. [CrossRef]

40. Jasper, M.J.; Tremellen, K.P.; Robertson, S.A. Primary unexplained infertility is associated with reduced expression of the T-regulatory cell transcription factor Foxp3 in endometrial tissue. *Mol. Hum. Reprod.* **2006**, *12*, 301–308. [CrossRef]

41. Kallikourdis, M.; Betz, A.G. Periodic accumulation of regulatory T cells in the uterus: Preparation for the implantation of a semi-allogeneic fetus? *PLoS ONE* **2007**, *2*, e382. [CrossRef]

42. Bulun, S.E.; Cheng, Y.H.; Yin, P.; Imir, G.; Utsunomiya, H.; Attar, E.; Innes, J.; Julie Kim, J. Progesterone resistance in endometriosis: Link to failure to metabolize estradiol. *Mol. Cell. Endocrinol.* **2006**, *248*, 94–103. [CrossRef]

43. Burney, R.O.; Talbi, S.; Hamilton, A.E.; Vo, K.C.; Nyegaard, M.; Nezhat, C.R.; Lessey, B.A.; Giudice, L.C. Gene expression analysis of endometrium reveals progesterone resistance and candidate susceptibility genes in women with endometriosis. *Endocrinology* **2007**, *148*, 3814–3826. [CrossRef]

44. Savaris, R.F.; Groll, J.M.; Young, S.L.; DeMayo, F.J.; Jeong, J.W.; Hamilton, A.E.; Giudice, L.C.; Lessey, B.A. Progesterone resistance in PCOS endometrium: A microarray analysis in clomiphene citrate-treated and artificial menstrual cycles. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1737–1746. [CrossRef]

45. Usadi, R.S.; Groll, J.M.; Lessey, B.A.; Lininger, R.A.; Zaino, R.J.; Fritz, M.A.; Young, S.L. Endometrial development and function in experimentally induced luteal phase deficiency. *J. Clin. Endocrinol. Metab.* **2008**, *93*, 4058–4064. [CrossRef] [PubMed]

46. Quezada, S.; Avellaira, C.; Johnson, M.C.; Gabler, F.; Fuentes, A.; Vega, M. Evaluation of steroid receptors, coregulators, and molecules associated with uterine receptivity in secretory endometria from untreated women with polycystic ovary syndrome. *Fertil. Steril.* **2006**, *85*, 1017–1026. [CrossRef] [PubMed]

47. Paulson, M.; Sahlin, L.; Hirschberg, A.L. Progesterone Receptors and Proliferation of the Endometrium in Obese Women With Polycystic Ovary Syndrome-A Lifestyle Intervention Study. *J. Clin. Endocrinol. Metab.* **2017**, *102*, 1244–1253. [CrossRef] [PubMed]

48. Hu, M.; Li, J.; Zhang, Y.; Li, X.; Brannstrom, M.; Shao, L.R.; Billig, H. Endometrial progesterone receptor isoforms in women with polycystic ovary syndrome. *Am. J. Transl. Res.* **2018**, *10*, 2696–2705.

49. Gregory, C.W.; Wilson, E.M.; Apparao, K.B.; Lininger, R.A.; Meyer, W.R.; Kowalik, A.; Fritz, M.A.; Lessey, B.A. Steroid receptor coactivator expression throughout the menstrual cycle in normal and abnormal endometrium. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2960–2966. [CrossRef] [PubMed]

50. Chrousos, G.P.; MacLusky, N.J.; Brandon, D.D.; Tomita, M.; Renquist, D.M.; Loriaux, D.L.; Lipsett, M.B. Progesterone resistance. *Adv. Exp. Med. Biol.* **1986**, *196*, 317–328. [CrossRef]

51. Igarashi, T.M.; Bruner-Tran, K.L.; Yeaman, G.R.; Lessey, B.A.; Edwards, D.P.; Eisenberg, E.; Osteen, K.G. Reduced expression of progesterone receptor-B in the endometrium of women with endometriosis and in cocultures of endometrial cells exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Fertil. Steril.* **2005**, *84*, 67–74. [CrossRef] [PubMed]

52. DeMayo, F.J.; Lydon, J.P. 90 YEARS OF PROGESTERONE: New insights into progesterone receptor signaling in the endometrium required for embryo implantation. *J. Mol. Endocrinol.* **2020**, *65*, T1–T14. [CrossRef] [PubMed]

53. Storer, C.L.; Dickey, C.A.; Galigniana, M.D.; Rein, T.; Cox, M.B. FKBP51 and FKBP52 in signaling and disease. *Trends Endocrinol. Metab.* **2011**, *22*, 481–490. [CrossRef]

54. Carrello, A.; Allan, R.K.; Morgan, S.L.; Owen, B.A.L.; Mok, D.; Ward, B.K.; Minchin, R.F.; Toft, D.O.; Ratajczak, T. Interaction of the Hsp90 cochaperone cyclophilin 40 with Hsc70. *Cell Stress Chaperones* **2004**, *9*, 167–181. [CrossRef]

55. Cheung-Flynn, J.; Roberts, P.J.; Riggs, D.L.; Smith, D.F. C-terminal sequences outside the tetratricopeptide repeat domain of FKBP51 and FKBP52 cause differential binding to hsp90. *J. Biol. Chem.* **2003**, *278*, 17388–17394. [CrossRef]

56. Cox, M.B.; Riggs, D.L.; Hessling, M.; Schumacher, F.; Buchner, J.; Smith, D.F. FK506-binding protein 52 phosphorylation: A potential mechanism for regulating steroid hormone receptor activity. *Mol. Endocrinol.* **2007**, *21*, 2956–2967. [CrossRef] [PubMed]

57. Li, X.; O'Malley, B.W. Unfolding the action of progesterone receptors. *J. Biol. Chem.* **2003**, *278*, 39261–39264. [CrossRef]

58. Silverstein, A.M.; Galigniana, M.D.; Kanelakis, K.C.; Radanyi, C.; Renoir, J.M.; Pratt, W.B. Different regions of the immunophilin FKBP52 determine its association with the glucocorticoid receptor, hsp90, and cytoplasmic dynein. *J. Biol. Chem.* **1999**, *274*, 36980–36986. [CrossRef]

59. Lonard, D.M.; Kumar, R.; O'Malley, B.W. Minireview: The SRC family of coactivators: An entree to understanding a subset of polygenic diseases? *Mol. Endocrinol.* **2010**, *24*, 279–285. [CrossRef]

60. Rowan, B.G.; O'Malley, B.W. Progesterone receptor coactivators. *Steroids* **2000**, *65*, 545–549. [CrossRef]

61. Tranguch, S.; Cheung-Flynn, J.; Daikoku, T.; Prapapanich, V.; Cox, M.B.; Xie, H.R.; Wang, H.B.; Das, S.K.; Smith, D.F.; Dey, S.K. Cochaperone immunophilin FKBP52 is critical to uterine receptivity for embryo implantation. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 14326–14331. [CrossRef]

62. Hubler, T.R.; Denny, W.B.; Valentine, D.L.; Cheung-Flynn, J.; Smith, D.F.; Scammell, J.G. The FK506-binding immunophilin FKBP51 is transcriptionally regulated by progestin and attenuates progestin responsiveness. *Endocrinology* **2003**, *144*, 2380–2387. [CrossRef] [PubMed]

63. Vasquez, Y.M.; Wang, X.; Wetendorf, M.; Franco, H.L.; Mo, Q.; Wang, T.; Lanz, R.B.; Young, S.L.; Lessey, B.A.; Spencer, T.E.; et al. FOXO1 regulates uterine epithelial integrity and progesterone receptor expression critical for embryo implantation. *PLoS Genet.* **2018**, *14*, e1007787. [CrossRef] [PubMed]

64. Whitaker, L.H.; Murray, A.A.; Matthews, R.; Shaw, G.; Williams, A.R.; Saunders, P.T.; Critchley, H.O. Selective progesterone receptor modulator (SPRM) ulipristal acetate (UPA) and its effects on the human endometrium. *Hum. Reprod.* **2017**, *32*, 531–543. [CrossRef] [PubMed]

65. Barthel, A.; Schmoll, D.; Unterman, T.G. FoxO proteins in insulin action and metabolism. *Trends Endocrinol. Metab.* **2005**, *16*, 183–189. [CrossRef] [PubMed]

66. Huang, H.; Tindall, D.J. Dynamic FoxO transcription factors. *J. Cell Sci.* **2007**, *120*, 2479–2487. [CrossRef] [PubMed]

67. Li, N.; Wang, X.; Wang, X.; Yu, H.; Lin, L.; Sun, C.; Liu, P.; Chu, Y.; Hou, J. Upregulation of FoxO 1 Signaling Mediates the Proinflammatory Cytokine Upregulation in the Macrophage from Polycystic Ovary Syndrome Patients. *Clin. Lab.* **2017**, *63*, 301–311. [CrossRef]

68. Kohan, K.; Carvajal, R.; Gabler, F.; Vantman, D.; Romero, C.; Vega, M. Role of the transcriptional factors FOXO1 and PPARG on gene expression of SLC2A4 in endometrial tissue from women with polycystic ovary syndrome. *Reproduction* **2010**, *140*, 123–131. [CrossRef]

69. Hulchiy, M.; Nybacka, A.; Sahlin, L.; Hirschberg, A.L. Endometrial Expression of Estrogen Receptors and the Androgen Receptor in Women with Polycystic Ovary Syndrome: A Lifestyle Intervention Study. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 561–571. [CrossRef]

70. The Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Hum. Reprod.* **2008**, *23*, 462–477. [CrossRef]

71. Bansal, S.; Goyal, M.; Sharma, C.; Shekhar, S. Letrozole versus clomiphene citrate for ovulation induction in anovulatory women with polycystic ovarian syndrome: A randomized controlled trial. *Int. J. Gynaecol. Obstet.* **2020**. [CrossRef]

72. Baruah, J.; Roy, K.K.; Rahman, S.M.; Kumar, S.; Sharma, J.B.; Karmakar, D. Endometrial effects of letrozole and clomiphene citrate in women with polycystic ovary syndrome using spiral artery Doppler. *Arch. Gynecol. Obstet.* **2009**, *279*, 311–314. [CrossRef]

73. Bates, G.W., Jr.; Propst, A.M. Polycystic ovarian syndrome management options. *Obset. Gynecol. Clin.* **2012**, *39*, 495–506. [CrossRef]

74. Palomba, S. Aromatase inhibitors for ovulation induction. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 1742–1747. [CrossRef] [PubMed]

75. Teede, H.; Tassone, E.C.; Piltonen, T.; Malhotra, J.; Mol, B.W.; Pena, A.; Witchel, S.F.; Joham, A.; McAllister, V.; Romualdi, D.; et al. Effect of the combined oral contraceptive pill and/or metformin in the management of polycystic ovary syndrome: A systematic review with meta-analyses. *Clin. Endocrinol.* **2019**, *91*, 479–489. [CrossRef] [PubMed]

76. Palomba, S.; Falbo, A.; Zullo, F.; Orio, F., Jr. Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: A comprehensive review. *Endocr. Rev.* **2009**, *30*, 1–50. [CrossRef] [PubMed]

77. Balen, A.H.; Morley, L.C.; Misso, M.; Franks, S.; Legro, R.S.; Wijeyaratne, C.N.; Stener-Victorin, E.; Fauser, B.C.; Norman, R.J.; Teede, H. The management of anovulatory infertility in women with polycystic ovary syndrome: An analysis of the evidence to support the development of global WHO guidance. *Hum. Reprod. Update* **2016**, *22*, 687–708. [CrossRef] [PubMed]

78. Goodman, N.F.; Cobin, R.H.; Futterweit, W.; Glueck, J.S.; Legro, R.S.; Carmina, E. American Association of Clinical Endocrinologists, American College of Endocrinology, and Androgen Excess and Pcos Society Disease State Clinical Review: Guide to the Best Practices in the Evaluation and Treatment of Polycystic Ovary Syndrome—Part 2. *Endocr. Pract.* **2015**, *21*, 1415–1426. [CrossRef]

79. Christakou, C.; Kollias, A.; Piperi, C.; Katsikis, I.; Panidis, D.; Diamanti-Kandarakis, E. The benefit-to-risk ratio of common treatments in PCOS: Effect of oral contraceptives versus metformin on atherogenic markers. *Hormones* **2014**, *13*, 488–497. [CrossRef]

80. Yousuf, S.D.; Rashid, F.; Mattoo, T.; Shekhar, C.; Mudassar, S.; Zargar, M.A.; Ganie, M.A. Does the Oral Contraceptive Pill Increase Plasma Intercellular Adhesion Molecule-1, Monocyte Chemoattractant Protein-1, and Tumor Necrosis Factor-alpha Levels in Women with Polycystic Ovary Syndrome: A Pilot Study. *J. Pediatr. Adolesc. Gynecol.* **2017**, *30*, 58–62. [CrossRef]

81. Manzoor, S.; Ganie, M.A.; Amin, S.; Shah, Z.A.; Bhat, I.A.; Yousuf, S.D.; Jeelani, H.; Kawa, I.A.; Fatima, Q.; Rashid, F. Oral contraceptive use increases risk of inflammatory and coagulatory disorders in women with Polycystic Ovarian Syndrome: An observational study. *Sci. Rep.* **2019**, *9*, 10182. [CrossRef] [PubMed]

82. Ali, D.E.; Shah, M.; Ali, A.; Malik, M.O.; Rehman, F.; Badshah, H.; Ehtesham, E.; Vitale, S.G. Treatment with Metformin and Combination of Metformin Plus Pioglitazone on Serum Levels of IL-6 and IL-8 in Polycystic Ovary Syndrome: A Randomized Clinical Trial. *Horm. Metab. Res.* **2019**, *51*, 714–722. [CrossRef] [PubMed]

83. Morley, L.C.; Tang, T.; Yasmin, E.; Norman, R.J.; Balen, A.H. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst. Rev.* **2017**, *11*, CD003053. [CrossRef]

84. American Diabetes, A. 13. Management of Diabetes in Pregnancy. *Diabetes Care* **2017**, *40*, S114–S119. [CrossRef] [PubMed]

85. Vanky, E.; Stridsklev, S.; Heimstad, R.; Romundstad, P.; Skogoy, K.; Kleggetveit, O.; Hjelle, S.; von Brandis, P.; Eikeland, T.; Flo, K.; et al. Metformin versus placebo from first trimester to delivery in polycystic ovary syndrome: A randomized, controlled multicenter study. *J. Clin. Endocrinol. Metab.* **2010**, *95*, E448–E455. [CrossRef]

86. De Leo, V.; Musacchio, M.C.; Piomboni, P.; Di Sabatino, A.; Morgante, G. The administration of metformin during pregnancy reduces polycystic ovary syndrome related gestational complications. *Eur. J. Obset. Gynecol. Reprod. Biol.* **2011**, *157*, 63–66. [CrossRef] [PubMed]

87. Kumar, P.; Khan, K. Effects of metformin use in pregnant patients with polycystic ovary syndrome. *J. Hum. Reprod. Sci.* **2012**, *5*, 166–169. [CrossRef]

88. Lord, J.M.; Flight, I.H.; Norman, R.J. Insulin-sensitising drugs (metformin, troglitazone, rosiglitazone, pioglitazone, D-chiro-inositol) for polycystic ovary syndrome. *Cochrane Database Syst. Rev.* **2003**, CD003053. [CrossRef]

89. Hu, M.; Zhang, Y.; Feng, J.; Xu, X.; Zhang, J.; Zhao, W.; Guo, X.; Li, J.; Vestin, E.; Cui, P.; et al. Uterine progesterone signaling is a target for metformin therapy in PCOS-like rats. *J. Endocrinol.* **2018**, *237*, 123–137. [CrossRef]

90. Albaghdadi, A.J.; Hewitt, M.A.; Putos, S.M.; Wells, M.; Ozolins, T.R.; Kan, F.W. Tacrolimus in the prevention of adverse pregnancy outcomes and diabetes-associated embryopathies in obese and diabetic mice. *J. Transl. Med.* **2017**, *15*, 32. [CrossRef]

91. Zhang, Y.; Hu, M.; Meng, F.; Sun, X.; Xu, H.; Zhang, J.; Cui, P.; Morina, N.; Li, X.; Li, W.; et al. Metformin Ameliorates Uterine Defects in a Rat Model of Polycystic Ovary Syndrome. *Ebiomedicine* **2017**, *18*, 157–170. [CrossRef] [PubMed]

92. Zhai, J.; Yao, G.D.; Wang, J.Y.; Yang, Q.L.; Wu, L.; Chang, Z.Y.; Sun, Y.P. Metformin Regulates Key MicroRNAs to Improve Endometrial Receptivity Through Increasing Implantation Marker Gene Expression in Patients with PCOS Undergoing IVF/ICSI. *Reprod. Sci.* **2019**, *26*, 1439–1448. [CrossRef] [PubMed]

93. Zhang, X.Q.; Zhao, D.; Ma, Y.D.; Wang, Y.C.; Zhang, L.X.; Guo, W.J.; Zhang, J.H.; Nie, L.; Yue, L.M. Impact of Disturbed Glucose Homeostasis Regulated by AMPK in Endometrium on Embryo Implantation in Diabetes Mice. *Reprod. Sci.* **2020**, *27*, 1752–1757. [CrossRef] [PubMed]

94. Xiong, F.; Xiao, J.; Bai, Y.; Zhang, Y.; Li, Q.; Lishuang, X. Metformin inhibits estradiol and progesterone-induced decidualization of endometrial stromal cells by regulating expression of progesterone receptor, cytokines and matrix metalloproteinases. *Biomed. Pharm.* **2019**, *109*, 1578–1585. [CrossRef]

95. Zhang, L.; Liao, Q. Effects of testosterone and metformin on glucose metabolism in endometrium. *Fertil. Steril.* **2010**, *93*, 2295–2298. [CrossRef]

96. Li, X.; Cui, P.; Jiang, H.Y.; Guo, Y.R.; Pishdari, B.; Hu, M.; Feng, Y.; Billig, H.; Shao, R. Reversing the reduced level of endometrial GLUT4 expression in polycystic ovary syndrome: A mechanistic study of metformin action. *Am. J. Transl. Res.* **2015**, *7*, 574–586.

97. Usadi, R.S.; Legro, R.S. Reproductive impact of polycystic ovary syndrome. *Curr. Opin. Endocrinol. Diabetes Obes.* **2012**, *19*, 505–511. [CrossRef] [PubMed]

98. Birch Petersen, K.; Pedersen, N.G.; Pedersen, A.T.; Lauritsen, M.P.; la Cour Freiesleben, N. Mono-ovulation in women with polycystic ovary syndrome: A clinical review on ovulation induction. *Reprod. Biomed. Online* **2016**, *32*, 563–583. [CrossRef]

99. Xiao, J.; Chen, S.; Zhang, C.; Chang, S. The effectiveness of metformin ovulation induction treatment in patients with PCOS: A systematic review and meta-analysis. *Gynecol. Endocrinol.* **2012**, *28*, 956–960. [CrossRef]

100. Bordewijk, E.M.; Wang, R.; van Wely, M.; Costello, M.F.; Norman, R.J.; Teede, H.; Gurrin, L.C.; Mol, B.W.; Li, W. To share or not to share data: How valid are trials evaluating first-line ovulation induction for polycystic ovary syndrome? *Hum. Reprod. Update* **2020**, *26*, 929–941. [CrossRef] [PubMed]

101. Hyer, S.; Balani, J.; Shehata, H. Metformin in Pregnancy: Mechanisms and Clinical Applications. *Int. J. Mol. Sci.* **2018**, 19. [CrossRef]

102. Doi, S.A.R.; Furuya-Kanamori, L.; Toft, E.; Musa, O.A.H.; Islam, N.; Clark, J.; Thalib, L. Metformin in pregnancy to avert gestational diabetes in women at high risk: Meta-analysis of randomized controlled trials. *Obes. Rev.* **2020**, *21*, e12964. [CrossRef]

103. Van Weelden, W.; Wekker, V.; de Wit, L.; Limpens, J.; Ijas, H.; van Wassenaer-Leemhuis, A.G.; Roseboom, T.J.; van Rijn, B.B.; DeVries, J.H.; Painter, R.C. Long-Term Effects of Oral Antidiabetic Drugs During Pregnancy on Offspring: A Systematic Review and Meta-analysis of Follow-up Studies of RCTs. *Diabetes* **2018**, *9*, 1811–1829. [CrossRef]

104. Sharief, M.; Nafee, N.R. Comparison of letrazole and clomiphene citrate in women with polycystic ovaries undergoing ovarian stimulation. *J. Pak. Med. Assoc.* **2015**, *65*, 1149–1152. [PubMed]

105. Jovanovic, V.P.; Kort, D.H.; Guarnaccia, M.M.; Sauer, M.V.; Lobo, R.A. Does the addition of clomiphene citrate or letrazole to gonadotropin treatment enhance the oocyte yield in poor responders undergoing IVF? *J. Assist. Reprod. Genet.* **2011**, *28*, 1067–1072. [CrossRef] [PubMed]

106. Franik, S.; Eltrop, S.M.; Kremer, J.A.; Kiesel, L.; Farquhar, C. Aromatase inhibitors (letrozole) for subfertile women with polycystic ovary syndrome. *Cochrane Database Syst. Rev.* **2018**, *5*, CD010287. [CrossRef] [PubMed]

107. Mehdinejadiani, S.; Amidi, F.; Mehdizadeh, M.; Barati, M.; Safdarian, L.; Aflatoonian, R.; Alyasin, A.; Aghahosseini, M.; Pazhohan, A.; Hayat, P.; et al. The effects of letrozole and clomiphene citrate on ligands expression of Wnt3, Wnt7a, and Wnt8b in proliferative endometrium of women with Polycystic ovarian syndrome. *Gynecol. Endocrinol.* **2018**, *34*, 775–780. [CrossRef] [PubMed]

108. Xie, H.; Tranguch, S.; Jia, X.; Zhang, H.; Das, S.K.; Dey, S.K.; Kuo, C.J.; Wang, H. Inactivation of nuclear Wnt-beta-catenin signaling limits blastocyst competency for implantation. *Development* **2008**, *135*, 717–727. [CrossRef]

109. Weiss, N.S.; Kostova, E.; Nahuis, M.; Mol, B.W.J.; van der Veen, F.; van Wely, M. Gonadotrophins for ovulation induction in women with polycystic ovary syndrome. *Cochrane Database Syst. Rev.* **2019**, *1*, CD010290. [CrossRef] [PubMed]

110. Diamond, M.P.; Legro, R.S.; Coutifaris, C.; Alvero, R.; Robinson, R.D.; Casson, P.; Christman, G.M.; Ager, J.; Huang, H.; Hansen, K.R.; et al. Letrozole, Gonadotropin, or Clomiphene for Unexplained Infertility. *N. Engl. J. Med.* **2015**, *373*, 1230–1240. [CrossRef]

111. Wang, E.T.; Diamond, M.P.; Alvero, R.; Casson, P.; Christman, G.M.; Coutifaris, C.; Hansen, K.R.; Sun, F.; Legro, R.S.; Robinson, R.D.; et al. Androgenicity and fertility treatment in women with unexplained infertility. *Fertil. Steril.* **2020**, *113*, 636–641. [CrossRef]

112. Brown, J.; Farquhar, C.; Beck, J.; Boothroyd, C.; Hughes, E. Clomiphene and anti-oestrogens for ovulation induction in PCOS. *Cochrane Database Syst. Rev.* **2009**, CD002249. [CrossRef]

113. Kousta, E.; White, D.M.; Franks, S. Modern use of clomiphene citrate in induction of ovulation. *Hum. Reprod. Updat.* **1997**, *3*, 359–365. [CrossRef]

114. Palomba, S.; Russo, T.; Orio, F.; Falbo, A.; Manguso, F.; Sammartino, A.; Tolino, A.; Colao, A.; Carmina, E.; Zullo, F. Uterine effects of clomiphene citrate in women with polycystic ovary syndrome: A prospective controlled study. *Hum. Reprod.* **2006**, *21*, 2823–2829. [CrossRef]

115. Tulandi, T.; Martin, J.; Al-Fadhli, R.; Kabli, N.; Forman, R.; Hitkari, J.; Librach, C.; Greenblatt, E.; Casper, R.F. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. *Fertil. Steril.* **2006**, *85*, 1761–1765. [CrossRef]

116. Sharma, S.; Ghosh, S.; Singh, S.; Chakravarty, A.; Ganesh, A.; Rajani, S.; Chakravarty, B.N. Congenital Malformations among Babies Born Following Letrozole or Clomiphene for Infertility Treatment. *PLoS ONE* **2014**, *9*, e108219. [CrossRef] [PubMed]

117. Kosmas, I.P.; Tatsioni, A.; Fatemi, H.M.; Kolibianakis, E.M.; Tournaye, H.; Devroey, P. Human chorionic gonadotropin administration vs. luteinizing monitoring for intrauterine insemination timing, after administration of clomiphene citrate: A meta-analysis. *Fertil. Steril.* **2007**, *87*, 607–612. [CrossRef]

118. Yonggang, H.; Xiaosheng, L.; Zhaoxia, H.; Yilu, C.; Jiqiang, L.; Huina, Z. Effects of human chorionic gonadotropin combined with clomiphene on Serum E2, FSH, LH and PRL levels in patients with polycystic ovarian syndrome. *Saudi J. Biol. Sci.* **2017**, *24*, 241–245. [CrossRef] [PubMed]

119. Bedaiwy, M.A.; Abdelaleem, M.A.; Hussein, M.; Mousa, N.; Brunengraber, L.N.; Casper, R.F. Hormonal, follicular and endometrial dynamics in letrozole-treated versus natural cycles in patients undergoing controlled ovarian stimulation. *Reprod. Biol. Endocrinol.* **2011**, *9*, 83. [CrossRef] [PubMed]

120. Wang, L.; Wen, X.; Lv, S.; Zhao, J.; Yang, T.; Yang, X. Comparison of endometrial receptivity of clomiphene citrate versus letrozole in women with polycystic ovary syndrome: A randomized controlled study. *Gynecol. Endocrinol.* **2019**, *35*, 1–4. [CrossRef]

121. Zhao, P.-L.; Zhang, Q.-F.; Yan, L.-Y.; Huang, S.; Chen, Y.; Qiao, J. Functional investigation on aromatase in endometrial hyperplasia in polycystic ovary syndrome cases. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 8975–8979. [CrossRef]

122. Shi, S.; Hong, T.; Jiang, F.; Zhuang, Y.; Chen, L.; Huang, X. Letrozole and human menopausal gonadotropin for ovulation induction in clomiphene resistance polycystic ovary syndrome patients. *Medicine* **2020**, *99*, e18383. [CrossRef]

123. Ragy, M.M.; Abdel-Hamid, H.A.; Toni, N.D. Pathophysiological changes in experimental polycystic ovary syndrome in female albino rats: Using either hemin or L -arginine. *J. Cell. Physiol.* **2019**, *234*, 8426–8435. [CrossRef]

124. Ito-Yamaguchi, A.; Suganuma, R.; Kumagami, A.; Hashimoto, S.; Yoshida-Komiya, H.; Fujimori, K. Effects of metformin on endocrine, metabolic milieus and endometrial expression of androgen receptor in patients with polycystic ovary syndrome. *Gynecol. Endocrinol.* **2015**, *31*, 44–47. [CrossRef]

125. Mohsen, I.A.; Elkattan, E.; Nabil, H.; Khattab, S. Effect of metformin treatment on endometrial vascular indices in anovulatory obese/overweight women with polycystic ovarian syndrome using three-dimensional power doppler ultrasonography. *J. Clin. Ultrasound* **2013**, *41*, 275–282. [CrossRef]

126. Abdalmageed, O.S.; Farghaly, T.A.; Abdelaleem, A.A.; Abdelmagied, A.E.; Ali, M.K.; Abbas, A.M. Impact of Metformin on IVF Outcomes in Overweight and Obese Women with Polycystic Ovary Syndrome: A Randomized Double-Blind Controlled Trial. *Reprod. Sci.* **2018**, *26*, 1336–1342. [CrossRef] [PubMed]

127. Kino, T.; Hatanaka, H.; Hashimoto, M.; Nishiyama, M.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H.; Imanaka, H. FK-506, a novel immunosuppressant isolated from a Streptomyces. I. Fermentation, isolation, and physico-chemical and biological characteristics. *J. Antibiot.* **1987**, *40*, 1249–1255. [CrossRef]

128. Albaghdadi, A.J.H.; Feeley, C.A.; Kan, F.W.K. Low-Dose Tacrolimus Prevents Dysregulated Peri-Conceptional Ovarian and Systemic Immune Cellular Homeostasis in Subjects with PCOS. *Sci. Rep.* **2019**, *9*, 6528. [CrossRef] [PubMed]

129. Nakagawa, K.; Ota, K.; Kuroda, K.; Hisano, M.; Sugiyama, R.; Yamaguchi, K.; Kwak-Kim, J. Immunosuppression with Tacrolimus Improved Reproductive Outcome of Women with Repeated Implantation Failure and Elevated Peripheral Blood Th1/Th2 Cell Ratios. *Am. J. Reprod. Immunol.* **2014**, *73*, 353–361. [CrossRef] [PubMed]

130. Ho, S.; Clipstone, N.; Timmermann, L.; Northrop, J.; Graef, I.; Fiorentino, D.; Nourse, J.; Crabtree, G.R. The Mechanism of Action of Cyclosporin A and FK506. *Clin. Immunol. Immunopathol.* **1996**, *80*, S40–S45. [CrossRef] [PubMed]

131. Shibasaki, F.; Price, E.R.; Milan, D.; McKeon, F. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. *Nat. Cell Biol.* **1996**, *382*, 370–373. [CrossRef]

132. Park, Y.-J.; Yoo, S.-A.; Kim, M.; Kim, W.-U. The Role of Calcium–Calcineurin–NFAT Signaling Pathway in Health and Autoimmune Diseases. *Front. Immunol.* **2020**, *11*, 195. [CrossRef] [PubMed]

133. Crabtree, G.R.; Schreiber, S.L. SnapShot: Ca^{2+}-Calcineurin-NFATSignaling. *Cell* **2009**, *138*, 210–210.e1. [CrossRef]

134. Sieber, M.; Baumgrass, R. Novel inhibitors of the calcineurin/NFATc hub—Alternatives to CsA and FK506? *Cell Commun. Signal.* **2009**, *7*, 25. [CrossRef]

135. Liu, J.; Farmer, J.D.; Lane, W.S.; Friedman, J.; Weissman, I.; Schreiber, S.L. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* **1991**, *66*, 807–815. [CrossRef]

136. Naranjo-Gómez, M.; Climent, N.; Cos, J.; Oliva, H.; Bofill, M.; Gatell, J.M.; Gallart, T.; Pujol-Borrell, R.; Borras, F.E. Tacrolimus treatment of plasmacytoid dendritic cells inhibits dinucleotide (CpG-)-induced tumour necrosis factor-alpha secretion. *Immunology* **2006**, *119*, 488–498. [CrossRef] [PubMed]

137. Sakuma, S.; Higashi, Y.; Sato, N.; Sasakawa, T.; Sengoku, T.; Ohkubo, Y.; Amaya, T.; Goto, T. Tacrolimus suppressed the production of cytokines involved in atopic dermatitis by direct stimulation of human PBMC system. (Comparison with steroids). *Int. Immunopharmacol.* **2001**, *1*, 1219–1226. [CrossRef]

138. Ghartey-Kwansah, G.; Li, Z.; Feng, R.; Wang, L.; Zhou, X.; Chen, F.Z.; Xu, M.M.; Jones, O.; Mu, Y.; Chen, S.; et al. Comparative analysis of FKBP family protein: Evaluation, structure, and function in mammals and Drosophila melanogaster. *BMC Dev. Biol.* **2018**, *18*, 7. [CrossRef]

139. Davies, T.H.; Ning, Y.-M.; Sanchez, E.R. Differential Control of Glucocorticoid Receptor Hormone-Binding Function by Tetra-tricopeptide Repeat (TPR) Proteins and the Immunosuppressive Ligand FK506†. *Biochemistry* **2005**, *44*, 2030–2038. [CrossRef] [PubMed]

140. Takeuchi, H.; Iwamoto, H.; Nakamura, Y.; Hirano, T.; Konno, O.; Kihara, Y.; Chiba, N.; Yokoyama, T.; Takano, K.; Toraishi, T.; et al. Synergistic Effects of Calcineurin Inhibitors and Steroids on Steroid Sensitivity of Peripheral Blood Mononuclear Cells. *Cell Med.* **2015**, *7*, 51–57. [CrossRef]

141. Shim, S.; Yuan, J.P.; Kim, J.Y.; Zeng, W.; Huang, G.; Milshteyn, A.; Kern, D.; Muallem, S.; Ming, G.-L.; Worley, P.F. Peptidyl-Prolyl Isomerase FKBP52 Controls Chemotropic Guidance of Neuronal Growth Cones via Regulation of TRPC1 Channel Opening. *Neuron* **2009**, *64*, 471–483. [CrossRef]

142. Davies, T.H.; Sánchez, E.R. FKBP52. *Int. J. Biochem. Cell Biol.* **2005**, *37*, 42–47. [CrossRef] [PubMed]

143. Czar, M.J.; Owens-Grillo, J.K.; Yem, A.W.; Leach, K.L.; Deibel, M.R.; Pratt, W.B.; Welsh, M.J. The hsp56 immunophilin component of untransformed steroid receptor complexes is localized both to microtubules in the cytoplasm and to the same nonrandom regions within the nucleus as the steroid receptor. *Mol. Endocrinol.* **1994**, *8*, 1731–1741. [CrossRef] [PubMed]

144. Galigniana, M.D.; Harrell, J.M.; Murphy, P.J.M.; Chinkers, M.; Radanyi, C.; Renoir, J.-M.; Zhang, M.; Pratt, W.B. Binding of hsp90-Associated Immunophilins to Cytoplasmic Dynein: Direct Binding and in Vivo Evidence that the Peptidylprolyl Isomerase Domain Is a Dynein Interaction Domain†. *Biochem.* **2002**, *41*, 13602–13610. [CrossRef]

145. Davies, T.H.; Ning, Y.-M.; Sánchez, E.R. A New First Step in Activation of Steroid Receptors. *J. Biol. Chem.* **2002**, *277*, 4597–4600. [CrossRef] [PubMed]

146. Albaghdadi, A.J.; Kan, F.W. Immunosuppression with tacrolimus improved implantation and rescued expression of uterine progesterone receptor and its co-regulators FKBP52 and PIASy at nidation in the obese and diabetic mice: Comparative studies with metformin. *Mol. Cell. Endocrinol.* **2018**, *460*, 73–84. [CrossRef] [PubMed]

147. Hannoun, Z.; Greenhough, S.; Jaffray, E.; Hay, R.T.; Hay, D.C. Post-translational modification by SUMO. *Toxicol.* **2010**, *278*, 288–293. [CrossRef]

148. Man, J.-H.; Li, H.-Y.; Zhang, P.-J.; Zhou, T.; He, K.; Pan, X.; Liang, B.; Li, A.-L.; Zhao, J.; Gong, W.-L.; et al. PIAS3 induction of PRB sumoylation represses PRB transactivation by destabilizing its retention in the nucleus. *Nucleic Acids Res.* **2006**, *34*, 5552–5566. [CrossRef]

149. Abdel-Hafiz, H.A.; Horwitz, K.B. Post-translational modifications of the progesterone receptors. *J. Steroid Biochem. Mol. Biol.* **2014**, *140*, 80–89. [CrossRef]

150. Daniel, A.R.; Gaviglio, A.L.; Czaplicki, L.M.; Hillard, C.J.; Housa, D.; Lange, C.A. The Progesterone Receptor Hinge Region Regulates the Kinetics of Transcriptional Responses Through Acetylation, Phosphorylation, and Nuclear Retention. *Mol. Endocrinol.* **2010**, *24*, 2126–2138. [CrossRef]

151. Ke, W.; Chen, C.; Luo, H.; Tang, J.; Zhang, Y.; Gao, W.; Yang, X.; Tian, Z.; Chang, Q.; Liang, Z. Histone Deacetylase 1 Regulates the Expression of Progesterone Receptor A During Human Parturition by Occupying the Progesterone Receptor A Promoter. *Reprod. Sci.* **2016**, *23*, 955–964. [CrossRef]

152. Cheng, M.H.; Nelson, L.M. Mechanisms and Models of Immune Tolerance Breakdown in the Ovary. *Semin. Reprod. Med.* **2011**, *29*, 308–316. [CrossRef]

153. Zhou, C.; Wu, J.; Borillo, J.; Torres, L.; McMahon, J.; Lou, Y.-H. Potential Roles of a Special CD8αα+ Cell Population and CC Chemokine Thymus-Expressed Chemokine in Ovulation Related Inflammation. *J. Immunol.* **2008**, *182*, 596–603. [CrossRef]

154. Arruvito, L.; Sanz, M.; Banham, A.H.; Fainboim, L. Expansion of CD4+CD25+and FOXP3+ Regulatory T Cells during the Follicular Phase of the Menstrual Cycle: Implications for Human Reproduction. *J. Immunol.* **2007**, *178*, 2572–2578. [CrossRef]

155. Nedoszytko, B.; Lange, M.; Sokołowska-Wojdyło, M.; Renke, J.; Trzonkowski, P.; Sobjanek, M.; Szczerkowska-Dobosz, A.; Niedoszytko, M.; Górska, A.; Romantowski, J.; et al. The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part I: Treg properties and functions. *Adv. Dermatol. Allergol.* **2017**, *4*, 285–294. [CrossRef]

156. Świst, K.; Pajtasz-Piasecka, E. Wpływ czynników transkrypcyjnych na różnicowanie limfocytów T CD4⁺. *Postępy Hig. Med. Doświadczalnej* **2011**, *65*, 414–426. [CrossRef]

157. Nasri, F.; Doroudchi, M.; Jahromi, B.N.; Gharesi-Fard, B. T Helper Cells Profile and CD4+CD25+Foxp3+Regulatory T Cells in Polycystic Ovary Syndrome. *Iran. J. Immunol. IJI* **2018**, *15*, 175–185.

158. Shen, Z.; Song, Q.; Chen, L.; Zhong, B.; Tang, S.; Hao, F. Bidirectional immunoregulation of calcineurin inhibitor tacrolimus on FOXP3 transcription? *Med. Hypotheses* **2011**, *76*, 178–180. [CrossRef]

159. Vaeth, M.; Feske, S. NFAT control of immune function: New Frontiers for an Abiding Trooper. *F1000Research* **2018**, *7*, 260. [CrossRef]

160. Clipstone, N.A.; Crabtree, G.R. Identification of calcineurin as a key signalling enzyme in T-lymphocyte activation. *Nat. Cell Biol.* **1992**, *357*, 695–697. [CrossRef]

161. Mantel, P.-Y.; Ouaked, N.; Rückert, B.; Karagiannidis, C.; Welz, R.; Blaser, K.; Schmidt-Weber, C.B. Molecular Mechanisms Underlying FOXP3 Induction in Human T Cells. *J. Immunol.* **2006**, *176*, 3593–3602. [CrossRef] [PubMed]

162. Lee, S.K.; Park, M.-J.; Jhun, J.Y.; Beak, J.-A.; Choi, J.W.; Rye, J.-Y.; Jang, J.W.; Bae, S.H.; Yoon, S.K.; Choi, H.J.; et al. Combination Treatment With Metformin and Tacrolimus Improves Systemic Immune Cellular Homeostasis by Modulating Treg and Th17 Imbalance. *Front. Immunol.* **2021**, *11*, 581728. [CrossRef] [PubMed]

163. Eteghadi, A.; Pak, F.; Ahmadpoor, P.; Jamali, S.; Karimi, M.; Yekaninejad, M.S.; Kokhaei, P.; Nafar, M.; Amirzargar, A.A. Th1, Th2, Th17 cell subsets in two different immunosuppressive protocols in renal allograft recipients (Sirolimus vs mycophenolate mofetil): A cohort study. *Int. Immunopharmacol.* **2019**, *67*, 319–325. [CrossRef] [PubMed]

164. Nakagawa, K.; Kwak-Kim, J.; Hisano, M.; Kasahara, Y.; Kuroda, K.; Sugiyama, R.; Yamaguchi, K. Obstetric and perinatal outcome of the women with repeated implantation failures or recurrent pregnancy losses who received pre- and post-conception tacrolimus treatment. *Am. J. Reprod. Immunol.* **2019**, *82*, e13142. [CrossRef]