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mTOR INHIBITORS AND RISK OF OVARIAN CYSTS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Abstract

Objective: Since the mTOR signaling pathway is known to regulate ovarian function, adverse events are likely to occur during treatment with mTOR inhibitors (mTORi). To summarize the available evidence on frequency of ovarian cyst development during mTORi treatment.

Methods: PubMed/MEDLINE and EMBASE databases were searched, from 1990 up to March 2020, using the following keywords: "tacrolimus", "sirolimus", "temsirolimus", "everolimus", "deforolimus", "mTOR" and "ovarian cysts" (Limit: Human, English, full article). Studies were selected for the review if they met all the following criteria: clinical studies, studies reporting original data, studies reporting the number of patients using mTORi, studies reporting the number of patients with ovarian cysts. Reviews, commentaries, and case reports were excluded from the review.

Two authors independently reviewed eligibility, extracted data, and assigned overall quality ratings based on predetermined criteria, with a third reviewer solving any disagreements. We selected 7 of 20 retrieved studies. Study design, population, sample size, criteria for diagnosis of ovarian cysts, drug doses and follow-up length were extracted. Pooled estimate of incidence was calculated for ovarian cysts as a percentage, with 95% confidence interval (CI).

Results: Four hundred-six women were included in the selected studies. The pooled incidence was 37.0% (95% CI 16.0-58.1%) for all ovarian cysts, and 17.3% (95% CI 5.6%-29.1%) for clinically significant ovarian cysts. Based on two articles, comparing mTORi and non-mTORi for immunosuppression, pooled odds ratio for ovarian cyst incidence was 4.62 (95% confidence interval 2.58-8.28).

Conclusion: In conclusion, ovarian cyst development is a common adverse event during immunosuppression treatment with mTOR inhibitors. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery. Based on these

considerations, women and physicians should be warned in the routine clinical practice about the gynecological impact of long-term use of mTOR inhibitors.

Strengths and limitations of this study

- Due to the widespread role of mTOR, mTORi may impact different organs and systems causing side effects that could be serious and/or debilitating.
- The mTOR signaling pathway is known to regulate ovarian function [2], thus it is conceivable that mTORi may affect ovarian activity.
- In the early 2000s, observational data have suggested that mTOR inhibitors, sirolimus in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.
- This study summarizes the available evidence on frequency of ovarian cyst development during mTORi treatment.
- Most studies included an extremely limited number of subjects and although meta-analyses provide an explicit method for synthesizing evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study.

Introduction

The mammalian target of rapamycin (mTOR) kinase regulates cell growth and metabolism in response to intra- and extracellular energetic stimuli and growth factors. The importance of mTOR in health and diseases has pushed the development of drugs that inhibit mTOR signaling (mTOR inhibitors, mTORi), including rapalogs, such as sirolimus (SRL), temsirolimus, tacrolimus (TAC), everolimus and deforolimus, which complex with FK506-binding protein 12 to inhibit mTOR complex 1 activity in an allosteric manner, or the more recent ATP-competitive mTORi (such as dactolisib), which targets the catalytic site of the enzyme [1].

Due to the widespread role of mTOR, mTORi may impact different organs and systems causing side effects that could be serious and/or debilitating. The mTOR signaling pathway is known to regulate ovarian function [2], thus it is conceivable that mTORi may affect ovarian activity. Along this line, in the early 2000s, observational data have suggested that mTOR inhibitors, sirolimus in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.

In this paper we reviewed the available data on the reported frequency of ovarian cysts, during treatment with mTORi sirolimus.

METHODS

We searched the PubMed (National Library of Medicine, Washington, DC) and EMBASE databases from 1990 up to March 2020 using different combinations of the following keywords: (a) “tacrolimus”, “sirolimus”, “temsirolimus”, “everolimus”, “deforolimus” and “mTOR” and “ovarian cysts” (Limit: Human, English, full article).

Furthermore, we reviewed reference lists of retrieved articles to search for other pertinent studies.

Two authors reviewed the papers and independently selected the articles eligible for the systematic review and extracted data. Any disagreements were submitted to a third reviewer to solve.

Inclusion criteria. Studies were selected for the review if they met all the following criteria: clinical studies, studies reporting original data, studies reporting number of patients using mTORi, studies reporting number of patients with ovarian cysts.

Exclusion criteria. Reviews, commentaries, and case reports were excluded from the review.

The present review and meta-analysis were conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guideline [3].

Patients and Public Involvement

It was not appropriate to involve patients or the public in our research.

Data extraction

A PICOS (Patient, Intervention, Comparator, Outcome, Study) design structure was used to develop the study questions and the inclusion/exclusion criteria. The question was, "Is there a relationship between mTORi sirolimus and ovarian cysts?" (Table 1).

Table 1. PICOS criteria for inclusion and exclusion of studies.

Parameter	**Inclusion criteria**	**Data extraction**
Patient	Women treated with mTOR Inhibitors	Location, age, type of patients
Intervention	mTOR Inhibitors	Dose and duration
Comparator	No treatment	Group definition
Outcome	Ovarian cysts yes/no	Number of cases, type of assessment
Study	Cross-sectional, cohort, case–control studies, clinical trials	Type of study design

For each study, the following information was extracted: first author's last name; year of publication; country of origin; design of the study; number of subjects treated with sirolimus; age if present; criteria for the diagnosis of ovarian cysts; type and dose of drug; length of follow-up; number of women with newly diagnosed ovarian cyst.

Quality Assessment

The quality of the studies included in the review was assessed using the Newcastle-Ottawa scale [4]. This instrument was developed to assess the quality of non-randomized studies, specifically cohort and case-control studies. Studies were judged based on three broad categories: selection of study groups, comparability of study groups, and assessment of outcome (cohort studies) or ascertainment of exposure (case-control studies). The maximum score was 9.

Randomized Controlled Trials (RCTs) were evaluated using the Revised Cochrane risk-of-bias tool for randomized trials [5].

Data synthesis

The primary outcomes assessed were ovarian cyst (overall and clinically relevant as defined by authors) in the total series and, if available, separately for premenopausal and postmenopausal women.

For each study with binary outcomes, we calculated the 95% confidence intervals (CI) of the estimated proportion. To evaluate the association between ovarian malignancy and menopausal status, we computed Pearson Chi Square test for heterogeneity and relative p value.

We used Metaprop, a command implemented in Stata to compute meta-analysis of proportions (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). Freeman-Tukey method was applied to include, in the computation, the studies with outcome proportion equal zero [6].

Estimates of proportion and 95% CI were calculated by using random effect model. To evaluate heterogeneity among studies, heterogeneity chi square p value was also reported. We assessed the heterogeneity among studies using the $\chi 2$ test [7] and quantified it using the I2 statistic. Results were defined as heterogeneous for P values less than 0.10. We computed summary estimates for ovarian cysts. We also rerun the analysis excluding the most extreme result, to evaluate if the summary estimate substantially changed.

Results.

The initial search retrieved 16 abstracts from Pubmed, and 13 from Embase. Nine publications were retrieved both in Pubmed/MEDLINE and EMBASE and 11 were excluded after reviewing abstracts: five laboratory studies, three case reports, one did not include drugs of interest, and two were reviews. Thus, nine publications remained to be fully read [8–16]. One paper was excluded because it was duplicate [11] and another because the number of cases of ovarian cysts was not reported, although they were described as "very frequent" [16]. One paper [13] reported the update of a previous one [12]; thus, the latter [12] was excluded from the main analysis but included in the sub-analysis for menopausal status, since this information was missing in the updated report [13].

Figure 1 shows the flow diagram of the literature search results.

A total of six studies have been identified: they were conducted in samples of women with Type 1 Diabetes Mellitus (T1DM) who underwent allogeneic islet transplantation (AIT) [8,12,13], in women with polycystic kidney disease [10] and in renal transplant recipients [9,14,15]. Main methodological characteristics are presented in Table 2.

Table 2. Main characteristics of selected studies.

Authors	Study design	Population, country	Sample size	Criteria for diagnosis of ovarian cyst	Drug doses	Follow-up
Cure et al, 2004 updated by Del Olmo Garcia (2011)	Cohort study	Women with T1DM who underwent AIT U.S.A. or multicentric	13 SRL+TAC mean age 41.0 (SD 8.8) years	Pelvic US (>3.0 cm in diameter that did not resolve spontaneously over 4 months)	TAC serum levels of 3–6 ng/mL SRL levels of 12–15 ng/mL for the first 90 days and 7–12 ng/mL thereafter.	24 months
Gaber et al, 2008	RCT	high-risk renal allograft recipients U.S.A.	SRL+TAC: 104 SRL+CsA: 98 Age not reported separately for women	Not reported	SRL levels of 10-15 ng/mL TAC up to 0.2 mg/kg/day to achieve levels of 10 to 15 ng/mL between day 1 and week 2, 5-10 ng/mL between weeks 2 and 26, and 3-5 ng/mL between weeks 26 and 52	12 months
Alfadhli et al, 2009	retrospective chart review	Women with T1DM who underwent AIT Canada	SRL+TAC=57 women median age 42.5 44 (70.5%) pre-menopausal 13 (15.4%) post-menopausal	Pelvic US (>2.5 cm in diameter)	SRL (trough levels 12–15 ng/ml for the first 3 months then 7–10 ng/ml thereafter) and TAC (target trough level 3–6 ng/ml). TAC at higher doses (target trough levels 10 ng/ml) along with mycophenolate mofetil (1 g b.i.d. as tolerated)	median 53.1 IQR 32.0–70.4 months
Del Olmo Garcia et al, 2011	Cohort study	Women with T1DM who underwent AIT U.S.A. or multicentric	SRL=18 mean age 48.5 (SD 8.00) years	pelvic US	SRL: serum levels 12–15 ng/ml for the first 90 days and 7–12 ng/ml thereafter	mean 7.9 (SD 1.13) years

Braun M et al, 2012	RCT	Adult females with autosomal dominant polycystic kidney disease Switzerland	SRL= 21 (mean age 31) standard care= 18 (mean age 32)	MRI without contrast material (> 2 cm in diameter)	SRL 1.3 to 1.5 mg day	18 months
Ignjatovic et al, 2013	retrospective chart review	Renal transplant recipients Serbia	SRL=6 women converted from CNI Age not reported	Not reported	SRL: serum levels 7–10 ng/ml for months 6 to 12 after transplant, 5-10 ng/ml thereafter	mean 65 (SD 20) months
Bachmann et al, 2017	Retrospective chart review	Renal transplant recipients Germany	mTORi= 102 other treatments: 469 (median age 32 for OC patients)	Pelvic US	SRL or Everolimus (trough level 3–8 ng/mL)	41.9 months (range 4.5–307)

T1DM: Type 1 Diabetes Mellitus; AIT: allogeneic islet transplantation; US: ultrasound; TAC: tacrolimus; SRL: sirolimus; RCT: Randomized Clinical Trial; CsA: cyclosporine A; MRI: magnetic resonance imaging; OC: Ovarian cysts; CNI: chronic calcineurin inhibitor
IQR: interquartile range; SD: standard deviation

Three studies were retrospective chart review [8,9,15], one was a cohort study [12,13] and two were RCTs [10,14]. Three studies included women with T1DM who underwent allogenic islet transplantation [8,12,13], three kidney transplantation recipients [9,14,15] and one study enrolled women with autosomal dominant polycystic kidney disease [10].

Diagnosis of ovarian cysts was based on pelvic ultrasound examination in four studies [8,9,12,13] with magnetic resonance imaging (MRI) without contrast in one study [10], whereas two did not report the diagnostic criteria [14,15].

SRL was given at increasing dose to reach serum levels ranging from 7 to 15 ng/ml. In one study SRL was given at doses of 1.3 to 1.5 mg SRL per day. TAC target was level 3–6 ng/ ml when given in association with sirolimus and or 10 ng/ ml when used in association with mycophenolate mofetil (1 g b.i.d. as tolerated).

Overall, the considered studies included 406 women who received SRL alone or in combination with other drugs, with mean follow-up ranging from 12 to 95 months.

Quality of selected studies

Both Braun et al. [10c] and Gaber et al. [14] had low risk of bias according to the Cochrane risk of bias tool (Table 3).

Table 3. Study quality evaluation according the Newcastle-Ottawa Scale (cohort studies) or Cochrane risk of bias (randomized clinical trials).

Publications							
Cohort study		**Selection**		**Comparability**		**Outcome (CS)**	**Study quality §**
Cure et al, 2004	1 2 3 4	* - * *	1 2	* -	1 2 3	* * -	6/9
Alfadhli et al, 2009	1 2 3 4	* * * -	1 2	* -	1 2 3	* * *	7/9
Del Olmo Garcia et al, 2011	1 2 3 4	* - * *	1 2	- -	1 2 3	* * -	5/9

Ignjatovic et al, 2013	1 2 3 4	* - * *	1 2	- -	1 2 3	- * *	4/9
Bachmann et al, 2017	1 2 3 4	* * * *	1 2	* *	1 2 3	* * *	9/9
RCT							Overall risk of bias
Braun M et al 2012	Randomization: some concern Assignment to intervention: low risk Adhering to intervention: low risk Missing outcome: low risk Measure of outcome: low risk Selection of results: low risk						Low
Gaber et al, 2008	Randomization: low risk Assignment to intervention: low risk Adhering to intervention: low risk Missing outcome: low risk Measure of outcome: low risk Selection of results: low risk						Low

§ We used the Newcastle– Ottawa quality assessment scale for cohort studies with maximum score 9, as presented at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed April 24, 2017). Most items were evaluated as "-" because of the small sample size or absence of not exposed cohort.

For the assessment of randomized controlled studies, we used the revised Cochrane risk of bias tool as presented at https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2

As regards to observational cohorts, using the NOS tool study quality was deemed good (8 or 9 out of 9) in Bachmann et al.'s paper [9]. Alfadhli et al.'s study was of some concern because it was unclear if baseline ultrasound scans were detailed enough to identify ovarian cysts [8]. Del Olmo Garcia et al. [13] and Ignjatovic et al. [15] presented mainly descriptive articles, including 18 (13 of whom already included in the paper by Cure et al. [12]) and six women respectively. Therefore, the possibility of some NOS quality item evaluation was debatable (i.e., if sample size was too little to control for important factors or if a not exposed cohort did exist).

Main results

Table 4 reports the frequency of ovarian cysts in women treated with SRL, SRL+TAC and SRL or everolimus. Two studies [8,12] reported the frequency in strata of menopausal status, suggesting that premenopausal women were at higher risk of developing ovarian cysts during mTORi treatment.

Table 4. Results of selected studies: patients with incident ovarian cyst on the total of treated women.

	SRL	**SRL + TAC**	**SRL or everolimus**	**All**	**Standard treatment**
Total series					
Gaber et al, 2008	7/98*	1/104		8/202	
Alfadhli et al, 2009		33/57		33/57	
Del Olmo Garcia et al, 2011	10/18			10/18	
Ignjatovic et al, 2013	2/6			2/6	
Comparative studies					
Braun M et al 2012	12/21			12/21	5/18
Bachmann et al, 2017			21/102	21/102	23/469
Total	*31/143*	*34/161*	*21/102*	*86/406*	*28/487*
Pre-menopause					
Cure et al, 2004		7/9		7/9	
Alfadhli et al, 2009		31/44		31/44	
Total		*38/53*		*38/53*	

Post-menopause					
Cure et al, 2004		1/4		1/4	
Alfadhli et al, 2009		2/13		2/13	
Total		*3/17*		*3/17*	
Clinically significant					
Alfadhli et al, 2009		14/57			
Del Olmo Garcia et al, 2011	8/18				
Braun M et al 2012	1/21				0/18
Bachmann et al, 2017			10/102		8/487
Total	*9/39*	*14/57*	*10/102*		*8/505*

*SRL+Cyclosporine

Systematic review

Gaber et al. [14] conducted a RCT to evaluate the efficacy and safety of SRL plus TAC versus SRL plus cyclosporine (CsA) in high-risk renal allograft recipients. A total of 202 women were randomly assigned before transplant to receive SRL-TAC (104 women) or SRL-CsA (98 women) with corticosteroids. Patients randomly assigned to SRL-TAC received a 10-mg loading dose of SRL on days 1 and 2, and 5 mg once daily, thereafter, adjusted to achieve whole blood trough concentrations from 10 to 15 ng/mL (measured by high performance liquid chromatography methodology). Up to 0.2 mg/kg/day of TAC was administered in divided oral doses (twice daily) to achieve whole blood concentrations from 10 to 15 ng/mL between day 1 and week 2, from 5 to 10 ng/mL between weeks 2 and 26, and from 3 to 5 ng/mL between weeks 26 and 52 (measured by monoclonal TDx or equivalent methodology). Patients randomly assigned to SRL-CsA received a larger 15-mg loading

dose of SRL on day 1, and 5 mg once daily, thereafter, adjusted to achieve the same whole blood trough concentrations as the patients assigned to SRL-TAC. One case of ovarian cyst was observed in the SRL-TAC group (1.0%) and seven in the SRL-CsA group (7.1%) (p=0.031).

Alfadhli et al.[8] conducted a chart review retrospective study in 57 women who underwent islet transplantation and received maintenance immunosuppression with SRL (trough levels 12–15 ng/ml for the first 3 months then 7–10 ng/ml thereafter) and TAC (target trough level 3–6 ng/ ml). A small group of patients received TAC at higher doses (target trough levels 10 ng/ml) along with mycophenolate mofetil (1 g b.i.d. as tolerated) for immunosuppression from the time of transplant. Ovarian cysts were found in 33 out of 57 women at a median of 235 (119–405) days after the first islet transplantation: 31 out of 44 (70.5%) premenopausal and two out of 13 (15.4%) postmenopausal women (P = 0.001). Ovarian cysts occurred more frequently in subjects taking SRL plus TAC than those taking high doses of TAC plus mycophenolate mofetil (33/53, 62.3%, vs. 0/4, 0%, P = 0.027). No women using combined oral contraception developed ovarian cysts. Among women taking SRL, average SRL trough levels were similar between those who developed ovarian cysts and those who did not (median 12.1, interquartile range, IQR 10.9–13.3, vs. 12.2, IQR 11.5–12.6 ng/ml, P = 0.993). SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects. The median maximal cyst diameter was 6.0 (3.8–7.6) cm. Most cysts were asymptomatic and noted incidentally on routine imaging. However, 14 subjects (42.4%) reported pelvic pain. In four cases, severe pelvic pain resulted in emergency room visits because of ovarian cyst rupture (n = 2) or torsion (n = 2). Histology was benign in all cases.

Del Olmo Garcia et al. [13] reported a total of 18 subjects (mean age at transplantation 48.5, standard deviation, SD, 8.0 years) with T1DM, who underwent allogeneic transplantation and were treated with SRL, given orally pre-transplant, at 0.2 mg/kg, and then adjusted to achieve trough levels of 12–15ng/ml for the first 90 days and 7–12 ng/ml thereafter. After the transplant, they were followed for a mean time of 7.9 (SD 1.13) years. In this study, a total of ten ovarian cysts (56%) were observed. All the cysts were benign, but eight were considered complex: four women (40%) underwent

cystectomy because of poor response to medical treatment. Part of this sample (13 out of 18) was previously described by Cure et al. [12], who reported that in four women, postmenopausal at the time they were transplanted, one case of ovarian cyst was observed, whereas out of nine premenopausal women seven developed ovarian cysts.

Braun et al. [10] reviewed the occurrence of ovarian cysts in a post hoc analysis of an open label randomized controlled phase II trial, conducted between March 2006 and March 2010. Women with autosomal dominant polycystic kidney disease were treated with 1.3 to 1.5 mg SRL per day for a median of 19 months (N = 21) or standard care (N = 18). Ovarian cysts were observed in 12 out of 21 patients in the SRL group, compared to five out of 18 patients in the control group (hazard ratio 4.4, 95% confidence interval 1.1-26). Differences in ovarian cysts between SRL and control did not seem to depend on the contraceptive method (barrier methods: seven out of 11 and three out of nine patients in the SRL and control groups; oral contraceptives: five out of 10 and two out of nine patients in the SRL and control groups). Clinical significance of ovarian cysts was not reported.

Ignjiatovic et al.[15] reviewed 24 transplant patients (six women) who switched from calcineurin inhibitors (CNI) to SRL from 2003 to 2011. Patients converted from CNI to SRL, with target serum levels 7–10 ng/ml for months 6 to 12 after transplant, and 5-10 ng/ml thereafter. Early after the conversion, two patients developed ovarian cysts with oligomenorrhea and reconverted to CNI, with cyst resolution and return to regular period.

Bachmann et al. [9] compared the effect of mTOR inhibitors vs. non-mTOR inhibitor immunosuppression on the incidence, size and complication rate of ovarian cysts in renal transplant recipients. They retrospectively analyzed 571 consecutive female kidney transplant patients between 2000 and 2008; they were followed-up till December 2012. Of those, 102 (17.8%) patients received mTOR inhibitors for at least one month after transplantation. A total of 44 women (7.7%) with new ovarian cysts were reported, 21 among patients receiving mTOR inhibitors (20.5%) and 23 in the control group (4.9%). This difference was statistically significant ($p < 0.001$). The hospitalization rate

was also more frequent in the mTOR group, with 21 hospitalizations in ten mTORi patients versus nine hospitalizations in eight control subjects (p = 0.05).

Synthesis of results

Overall, 406 women were treated with mTORi in the six studies included in this meta-analysis and 86 developed ovarian cysts. The frequency of ovarian cysts in women treated with mTORi, without any specific restriction regarding the type of drug, is reported in Table 5.

Table 5. Pooled estimates of ovarian cyst incidence.

Authors	**Cases**	**Sample size**	**Pooled incidence estimate**	**95% confidence interval**
Gaber et al, 2008	8	202	4.0	2.0-7.6
Alfadhli et al, 2009	33	57	57.9	45.0-69.8
Del Olmo Garcia et al, 2011	10	18	55.6	33.7-75.4
Braun M et al, 2012	12	21	57.1	36.5-75.5
Ignjatovic et al, 2013	2	6	33.3	9.7-70.0
Bachmann et al, 2017	21	102	20.6	13.9-29.4
Random pooled estimate			37.0	16.0-58.1
Heterogeneity χ^2 = 115.0 (d.f. = 5) p = 0.0; I^2 (variation in ES attributable to heterogeneity) = 95.7% Estimate of between-study variance τ^2 = 0.1				
Random pooled estimate excluding Gaber et al.			44.9	23.7-66.1
Heterogeneity χ^2 = 32.5 (d.f. = 4) p = 0.0; I^2 (variation in ES attributable to heterogeneity) = 87.7% Estimate of between-study variance τ^2 = 0.1				

The pooled incidence was 37.0% (95% CI 16.0-58.1) (Figure 2). The rate of ovarian cysts ranged from 4.0% to 57.9%, leading to a remarkable heterogeneity (Chi-square for heterogeneity 115.0, $p<0.001$, $I^2=95.7\%$). Excluding the study with most extreme results [14] the pooled estimate increased to 44.9% (95% CI 23.7-67.1), with a small decrease of heterogeneity, that remained, however, remarkable.

Pooling the results of two comparative studies [8,12], we found that ovarian cyst rates were higher for premenopausal women (38/53, 71.7%) than postmenopausal ones (3/17, 17.6%) and the difference was statistically significant ($p<0.0001$): the odds ratio for developing ovarian cysts was 12.46 (95% confidence interval 3.04-50.98) comparing pre-menopausal with post-menopausal women.

Two studies compared mTOR inhibitors vs. non-mTOR inhibitor immunosuppression [9,10]. The pooled odds ratio for ovarian cyst incidence was 4.62 (95% confidence interval 2.58-8.28).

Lastly, we pooled the incidence of clinically significant ovarian cysts in studies reporting this information [8–10,12]. The resulting estimate was 17.3% (95% CI 5.6-29.1, heterogeneity chi-square 15.5, $p<0.001$).

Discussion.

This systematic review shows that, in women treated with mTOR inhibitors, the incidences of ovarian cysts ranged between less than 10% to more than 50%, in different studies and clinical series. The pooled incidence was 37%, 17% only considering clinically significant ovarian cysts. The risk seems to be higher among premenopausal women: two studies distinguished ovarian cyst incidence occurring in pre- and post-menopausal patients, with consistent results [8,12], suggesting that mTORi effect is higher in presence of spontaneous ovarian activity.

Where immunosuppression was achieved using mTOR inhibitors as compared to non-mTOR inhibitors [9,10], women on mTOR inhibitors were at higher risk of developing ovarian cysts.

The limited data and the differences in the presentation of results do not provide the opportunity of analyzing in detail the role of stopping mTORi on the clinical course of ovarian cysts or the protective role of oral contraceptive use.

In the study by Alfadhli et al. [8], SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects; however, the proportion with partial or complete cyst resolution was similar in those who did or did not discontinue SRL (12/15, 80%, vs. 6/8, 75%, P = NS).

Another potential risk factor for the development of ovarian cysts during mTORi use was a previous history of ovarian cysts; Del Olmo Garcia et al. [13] reported five patients with such a history.

In our synthesis, we found an extremely high heterogeneity, that may be due to both the different criteria for diagnosis of ovarian cysts and the type of disease requiring mTORi use. For example, T1DM is associated with menstrual irregularity and PCOS [17,18], hence a higher basal frequency of ovarian cyst development. Another factor likely affecting heterogeneity was the active screening for ovarian cysts development in women on mTORi treatment. In particular, it appears that in the study with the lowest incidence [14], women did not routinely undergo abdominal scans.

In biological terms, mTOR inhibitors may affect the levels of LH and FSH. Further, expression of progesterone receptors can be inhibited by sirolimus via the mTOR and inhibition of progesterone receptors in the ovaries may interfere with ovarian cysts development. However, the specific mechanisms linking mTOR inhibitor exposure and risk of developing ovarian cysts are unknown [12].

This review and meta-analysis may be affected by potential limitation or bias.

Findings from this systematic review and meta-analysis are based on an extremely limited number of studies, thus the results should be considered cautiously. Taking this aspect into account, the general results confirm clinical suggestion that mTORi increase the frequency of benign ovarian cysts.

Among studies, the heterogeneity was remarkable. This finding may be due to several characteristics of the selected samples. First, two studies included women with T1Dm who underwent allogeneic

islet transplantation [8,13], three included women who underwent renal transplantation [9,14,15] and one study women with autosomal dominant polycystic kidney [10]. Then, the ascertainment of ovarian cysts was performed with different methodologies, and in two studies the method was not reported. Lastly, the number of study participants was quite different among studies ranging between 6 and more than 200 women. Despite this, the pooled estimate is not overwhelmingly affected by the largest studies [9,14], and the study weights are similar (Figure 2).

We considered only publications published in English. Authors may be more prone to publish in an international, English-language journal if results are positive, whereas negative findings are more often published in local journals [19]. Limiting our analysis to publications in English language journals can therefore restrict the completeness of information, thereby causing bias. The direction and the strength of this bias are not however clear.

Another limitation is the fact that most of studies included an extremely limited number of subjects. Although systematic reviews with meta-analyses provide an explicit method for synthesizing evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study. Lastly, this study was not registered a priori.

Despite these limitations, consistent results among all studies give strong support to the general findings.

Although the biological and clinical explanation of the results of our analysis is not totally clear, observational studies and clinical trials consistently suggest that ovarian cysts are a common adverse effect of mTOR inhibitors. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery. Based on these considerations, women and physicians should be warned in routine clinical practice about the gynecological impact of long-term use of mTOR inhibitors.

Conflict of interest: all authors declare no conflict of interest.

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Authors' contribution

Stefano Bianchi, Sergio Harari, Fabio Parazzini and Sandro Gerli designed the study; Andrea Dell'Acqua, Alessandro Favilli and Michele Vignali reviewed the text; Fabio Parazzini, Elena Ricci and Francesca Chiaffarino performed the literature research and extracted the data; Sonia Cipriani and Elena Ricci performed the statistical analyses; Stefano Bianchi and Fabio Parazzini wrote the paper.

References

[1] Zheng Y, Jiang Y. mTOR Inhibitors at a Glance. Mol Cell Pharmacol 2015;7:15–20.

[2] Alam H, Maizels E, Park Y, Ghaey S, Feiger Z, Chandel N, et al. Follicle-stimulating hormone activation of hypoxia-inducible factor-1 by the phosphatidylinositol 3-kinase/AKT/Ras homolog enriched in brain (Rheb)/ mammalian target of rapamycin (mTOR) pathway is necessary for induction of select protein markers of follicular differentiation. J Biol Chem 2004;279:19431–40.

[3] Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med 2009;6:e1000097. https://doi.org/10.1371/journal.pmed.1000097.

[4] Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non randomised studies in meta-analyses n.d. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

[5] Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. Br Med J 2019;366:1–8. https://doi.org/10.1136/bmj.l4898.

[6] Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch Public Heal 2014;72:39.

[7] Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev 1987;9:1–30.

[8] Alfadhli E, Koh A, Albaker W, Bhargava R, Ackerman T, McDonald C, et al. High prevalence of ovarian cysts in premenopausal women receiving sirolimus and tacrolimus after clinical islet transplantation. Transpl Int 2009;22:622–5. https://doi.org/10.1111/j.1432-2277.2009.00839.x.

[9] Bachmann F, Glander P, Budde K, Bachmann C. High Incidence of Ovarian Cysts in Women Receiving mTOR Inhibitors after Renal Transplantation. J Women's Heal

2018;27:394–8. https://doi.org/10.1089/jwh.2017.6451.

[10] Braun M, Young J, Reiner CS, Poster D, Krauer F, Kistler AD, et al. Low-Dose Oral Sirolimus and the Risk of Menstrual-Cycle Disturbances and Ovarian Cysts: Analysis of the Randomized Controlled SUISSE ADPKD Trial. PLoS One 2012;7:e45868. https://doi.org/10.1371/journal.pone.0045868.

[11] Braun M, Young J, Reiner C, Poster D, Wüthrich R, Serra A. Ovarian toxicity from sirolimus. N Engl J Med 2012;366:1062–4.

[12] Cure P, Pileggi A, Froud T, Norris PM, Baidal DA, Cornejo A, et al. Alterations of the female reproductive system in recipients of islet grafts. Transplantation 2004;78:1576–81. https://doi.org/10.1097/01.TP.0000147301.41864.C0.

[13] Garcia MIDO, Lauriola V, Aracena AG, Messinger S, Corrales A, Ricordi C, et al. Alterations of the female reproductive system in islet recipient receiving immunosuppression. Cell Transplant 2011;20:1649–51. https://doi.org/10.3727/096368910x557209.

[14] Gaber AO, Kahan BD, Van Buren C, Schulman SL, Scarola J, Neylan JF. Comparison of sirolimus plus tacrolimus versus sirolimus plus cyclosporine in high-risk renal allograft recipients: Results from an open-label, randomized Trial. Transplantation 2008;86:1187–95. https://doi.org/10.1097/TP.0b013e318187bab0.

[15] Ignjatović L, Hrvacević R, Jovanović D, Kovacević Z, Vavić N, Rabrenović V, et al. Conversion from calcineurin inhibitors to sirolimus of recipients with chronic kidney graft disease grade III for a period 2003-2011. Vojn Pregl 2013;70:848–53.

[16] Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-Year Follow-Up After Clinical Islet Transplantation. Diabetes 2005;54:2060–9.

[17] Strotmeyer ES, Steenkiste AR, Foley TP, Berga SL, Dorman JS. Menstrual cycle differences between women with type 1 diabetes and women without diabetes. Diabetes Care 2003;26:1016–21. https://doi.org/10.2337/diacare.26.4.1016.

[18] Escobar-Morreale HF, Roldán B, Barrio R, Alonso M, Sancho J, De La Calle H, et al. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 2000;85:4182–7. https://doi.org/10.1210/jc.85.11.4182.

[19] Grégoire G, Derderian F, Le Lorier J. Selecting the language of the publications included in a meta-analysis: is there a Tower of Babel bias? J Clin Epidemiol 1995;48:159–63.

Figure 1. Flow chart of selected studies.

Figure 2. Forest plot of ovarian cyst incidence.

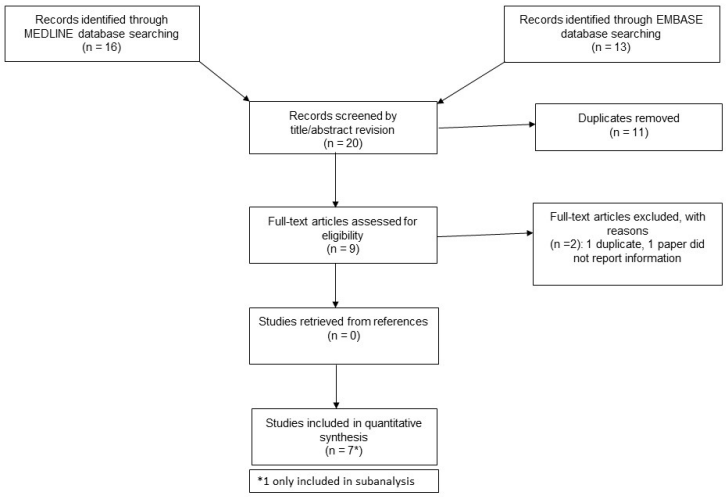

Figure 1

338x190mm (96 x 96 DPI)

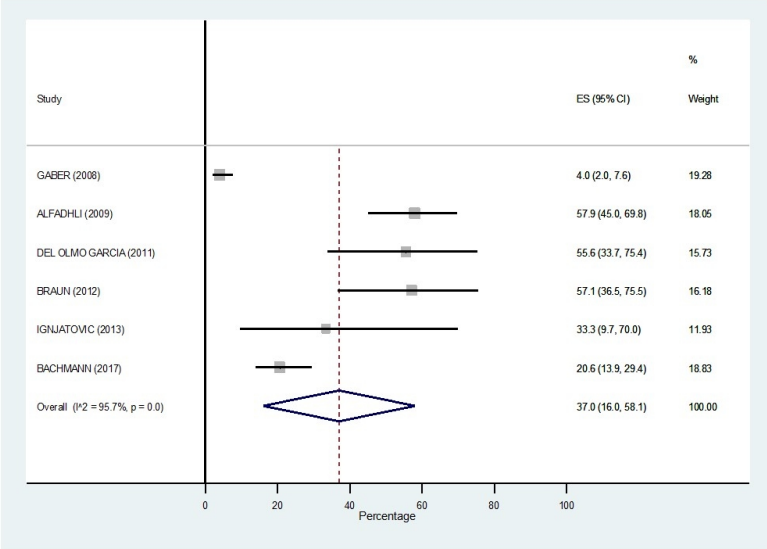

Figure 2

338x190mm (96 x 96 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6 Table 1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	-
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6 Table 1
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6-7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6-7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6-7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-16 Table 4 Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	17-18 Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	-
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	19-20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	21

PRISMA 2009 Checklist

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MTOR INHIBITORS AND RISK OF OVARIAN CYSTS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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mTOR INHIBITORS AND RISK OF OVARIAN CYSTS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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mTOR INHIBITORS AND RISK OF OVARIAN CYSTS: A SYSTEMATIC REVIEW AND META-ANALYSIS.

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Abstract

Objective: To summarize the available evidence on frequency of ovarian cyst development during mTORi treatment.

Methods: PubMed/MEDLINE and EMBASE databases were searched, from 1990 up to March 2020, using the following keywords: "tacrolimus", "sirolimus", "temsirolimus", "everolimus", "deforolimus", "mTOR" and "ovarian cysts" (Limit: Human, English, full article). Studies were selected for the review if they met all the following criteria: clinical studies, studies reporting original data, studies reporting the number of patients using mTORi, studies reporting the number of patients with ovarian cysts.

We selected 7 of 20 retrieved studies. Study design, population, sample size, criteria for diagnosis of ovarian cysts, drug doses and follow-up length were extracted. Pooled estimate of incidence was calculated for ovarian cysts as a percentage, with 95% confidence interval (CI).

Results: Four hundred-six women were included in the selected studies. The pooled incidence was 37.0% (95% CI 16.0-58.1%) for all ovarian cysts, and 17.3% (95% CI 5.6%-29.1%) for clinically significant ovarian cysts. Based on two articles, comparing mTORi and non-mTORi for immunosuppression, pooled odds ratio for ovarian cyst incidence was 4.62 (95% CI 2.58-8.28).

Conclusion: Ovarian cyst development is a common adverse event during immunosuppression treatment with mTOR inhibitors. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery

Strengths and limitations of this study

Due to the widespread role of mTOR, mTORi may impact different organs and systems causing side effects that could be serious and/or debilitating.

- The mTOR signaling pathway is known to regulate ovarian function, thus it is conceivable that mTORi may affect ovarian activity.
- In the early 2000s, observational data have suggested that mTOR inhibitors, sirolimus in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.
- This study summarizes the available evidence on frequency of ovarian cyst development during mTORi treatment.
- Most studies included an extremely limited number of subjects and although meta-analyses provide an explicit method for synthesizing evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study.

Introduction

The mammalian target of rapamycin (mTOR) kinase regulates cell growth and metabolism in response to intra- and extracellular energetic stimuli and growth factors. The importance of mTOR in health and diseases has pushed the development of drugs that inhibit mTOR signaling (mTOR inhibitors, mTORi), including rapalogs, such as sirolimus (SRL), temsirolimus, tacrolimus (TAC), everolimus and deforolimus, which complex with FK506-binding protein 12 to inhibit mTOR complex 1 activity in an allosteric manner, or the more recent ATP-competitive mTORi (such as dactolisib), which targets the catalytic site of the enzyme [1].

mTOR inhibitors are used as targeted therapy for tumors (in particular renal carcinoma). Further mTOR inhibitors inhibit T-cell proliferation and proliferative responses induced by several cytokines, including Interleukin 1, Interleukin 2, Interleukin 3, Interleukin 4, Interleukin 6, Insulin-like growth factor, Platelet-derived growth factor, and Colony-stimulating factors and they have been used in combination therapy with corticosteroids and cyclosporine in patients who received kidney transplantation to prevent organ rejection, and in the treatment of rheumatoid arthritis [1].

Due to the widespread role of mTOR, mTORi may impact different organs and systems causing side effects that could be serious and/or debilitating. The mTOR signaling pathway is known to regulate ovarian function [2], thus it is conceivable that mTORi may affect ovarian activity. Along this line, in the early 2000s, observational data have suggested that mTOR inhibitors, sirolimus in particular, may cause menstrual irregularities and high-volume ovarian cysts, needing surgical procedure.

In this paper we reviewed the available data on the reported frequency of ovarian cysts, during treatment with mTORi sirolimus.

METHODS

We searched the PubMed (National Library of Medicine, Washington, DC) and EMBASE databases from 1990 up to March 2020 using different combinations of the following keywords: (a)

"tacrolimus", "sirolimus", "temsirolimus", "everolimus", "deforolimus" and "mTOR" and "ovarian cysts" (Limit: Human, English, full article) (see Supplementary File 1).

Furthermore, we reviewed reference lists of retrieved articles to search for other pertinent studies.

Two authors reviewed the papers and independently selected the articles eligible for the systematic review and extracted data. Any disagreements were submitted to a third reviewer to solve.

Inclusion criteria. Studies were selected for the review if they met all the following criteria: clinical studies, studies reporting original data, studies reporting number of patients using mTORi, studies reporting number of patients with ovarian cysts.

Exclusion criteria. Reviews, commentaries, and case reports were excluded from the review.

The present review and meta-analysis were conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guideline [3].

Patients and Public Involvement

It was not appropriate to involve patients or the public in our research.

Data extraction

A PICOS (Patient, Intervention, Comparator, Outcome, Study) design structure was used to develop the study questions and the inclusion/exclusion criteria. The question was, "Is there a relationship between mTORi sirolimus and ovarian cysts?" (Table 1).

Table 1. PICOS criteria for inclusion and exclusion of studies.

Parameter	**Inclusion criteria**	**Data extraction**
Patient	Women treated with mTOR Inhibitors	Location, age, type of patients
Intervention	mTOR Inhibitors	Dose and duration
Comparator	No treatment	Group definition
Outcome	Ovarian cysts yes/no	Number of cases, type of assessment
Study	Cross-sectional, cohort, case–control studies, clinical trials	Type of study design

For each study, the following information was extracted: first author's last name; year of publication; country of origin; design of the study; number of subjects treated with sirolimus; age if present; criteria for the diagnosis of ovarian cysts; type and dose of drug; length of follow-up; number of women with newly diagnosed ovarian cyst. Further, we have collected information on the clinically significant ovarian cysts. This group includes symptomatic cysts, cyst >6cm and cysts requiring surgery (see below)

Quality Assessment

The quality of the studies included in the review was assessed using the Newcastle-Ottawa scale [4]. This instrument was developed to assess the quality of non-randomized studies, specifically cohort and case-control studies. Studies were judged based on three broad categories: selection of study groups, comparability of study groups, and assessment of outcome (cohort studies) or ascertainment of exposure (case-control studies). The maximum score was 9.

Randomized Controlled Trials (RCTs) were evaluated using the Revised Cochrane risk-of-bias tool for randomized trials [5].

Data synthesis

The primary outcomes assessed were ovarian cyst (overall and clinically relevant as defined by authors or requiring surgery) in the total series and, if available, separately for premenopausal and postmenopausal women.

For each study with binary outcomes, we calculated the 95% confidence intervals (CI) of the estimated proportion. To evaluate the association between ovarian malignancy and menopausal status, we computed Pearson Chi Square test for heterogeneity and relative p value.

We used Metaprop, a command implemented in Stata to compute meta-analysis of proportions (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP). Freeman-Tukey method was applied to include, in the computation, the studies with outcome proportion equal zero [6].

Estimates of proportion and 95% CI were calculated by using random effect model. To evaluate heterogeneity among studies, heterogeneity chi square p value was also reported. We assessed the heterogeneity among studies using the $\chi 2$ test [7] and quantified it using the I2 statistic. Results were defined as heterogeneous for P values less than 0.10. We computed summary estimates for ovarian cysts. We also rerun the analysis excluding the most extreme result, to evaluate if the summary estimate substantially changed.

Results.

The initial search retrieved 16 abstracts from Pubmed, and 13 from Embase. Nine publications were retrieved both in Pubmed/MEDLINE and EMBASE and 11 were excluded after reviewing abstracts: five laboratory studies, three case reports, one did not include drugs of interest, and two were reviews. Thus, nine publications remained to be fully read [8–16]. One paper was excluded because it was duplicate [11] and another because the number of cases of ovarian cysts was not reported, although they were described as "very frequent" [16]. One paper [13] reported the update of a previous one [12]; thus, the latter [12] was excluded from the main analysis but included in the sub-analysis for menopausal status, since this information was missing in the updated report [13].

Figure 1 shows the flow diagram of the literature search results.

A total of ~~six~~ seven studies have been identified: they were conducted in samples of women with Type 1 Diabetes Mellitus (T1DM) who underwent allogeneic islet transplantation (AIT) [8,12,13], in women with polycystic kidney disease [10] and in renal transplant recipients [9,14,15]. Main methodological characteristics are presented in Table 2.

Table 2. Main characteristics of selected studies.

Authors	Study design	Population, Country	Sample size	Criteria for diagnosis of ovarian cyst	Ovaries study performed before treatment	Drug doses	Follow-up	Definition of clinically significant ovarian cyst
Cure et al, 2004 [12] * updated by Del Olmo Garcia, 2011 [13]	Cohort study	Women with T1DM who underwent AIT U.S.A. or multicentric	SRL+TAC:13 mean age 41.0 (SD 8.8) years	Pelvic US (>3.0 cm in diameter that did not resolve spontaneously over 4 months)	See Olmo Garcia	TAC serum levels of 3–6 ng/mL SRL levels of 12–15 ng/mL for the first 90 days and 7–10 ng/mL thereafter.	24 months	>6 Four of the subjects (40%) underwent surgery
Gaber et al, 2008 [14]	RCT	High-risk renal allograft recipients U.S.A.	SRL+TAC: 104 SRL+CsA: 98 Age not reported separately for women	Not reported	Not reported	SRL levels of 10-15 ng/mL TAC up to 0.2 mg/kg/day to achieve levels of 10 to 15 ng/mL between day 1 and week 2, 5-10 ng/mL between weeks 2 and 26, and 3-5 ng/mL between	12 months	Not reported

						weeks 26 and 52		
Alfadhli et al, 2009 [8]	Retrospective chart review	Women with T1DM who underwent AIT Canada	SRL+TAC:57 women median age 42.5 44 (70.5%) pre-menopausal 13 (15.4%) post-menopausal	Pelvic US (>2.5 cm in diameter)	Routine pretransplant abdominal ultrasound scans	SRL (trough levels 12–15 ng/ml for the first 3 months then 7–10 ng/ml thereafter) and TAC (target trough level 3–6 ng/ml) TAC at higher doses (target trough levels 10 ng/ml) along with mycophenolate mofetil (1 g b.i.d. as tolerated)	median 53.1 IQR 32.0–70.4 months	However, 14 subjects (42.4%) reported pelvic pain. In four cases, severe pelvic pain resulted in emergency room visits because of ovarian cyst rupture (n = 2) or torsion (n = 2).
Del Olmo Garcia et al, 2011 [13]	Cohort study	Women with T1DM who underwent AIT U.S.A. or multicentric	SRL:18 mean age 48.5 (SD 8.00) years	Pelvic US	Peritransplant ultrasound examination	SRL serum levels 12–15 ng/ml for the first 90 days and 7–12 ng/ml thereafter	mean 7.9 (SD 1.13) years	See cure

Braun M et al, 2012 [11]	RCT	Adult females with autosomal dominant polycystic kidney disease Switzerland	SRL:21 (mean age 31) standard care= 18 (mean age 32)	MRI without contrast material (> 2 cm in diameter)	Abdominal magnetic resonance imaging (MRI) without contrast material	SRL: 1.3 to 1.5 mg day	18 months	One patient presented with acute abdominal pain and a large cyst of the left ovary while receiving sirolimus and was cystectomized at 164 days after randomization.
Ignjatovic et al, 2013 [15]	Retrospective chart review	Renal transplant recipients Serbia	SRL:6 women converted from CNI Age not reported	Not reported	Basic physical examination	SRL: serum levels 7–10 ng/ml for months 6 to 12 after transplant, 5-10 ng/ml thereafter	mean 65 (SD 20) months	Early after the conversion two of the patients developed serious crural edema and multiple ovarian cysts with oligomenorrhea. After reconversion to CNI they lost edema and ovarian cysts and returned to a regular period.
Bachmann et al, 2018 [9]	Retrospective chart review	Renal transplant recipients Germany	mTORi:102 other treatments: 469 (median age 32 for OC patients)	Pelvic US	Ultrasound examination in the early postoperative period (<4 weeks)	SRL or everolimus (trough level 3–8 ng/mL)	41.9 months (range 4.5–307)	Surgery

T1DM: Type 1 Diabetes Mellitus; AIT: allogeneic islet transplantation; US: ultrasound; TAC: tacrolimus; SRL: sirolimus; RCT: Randomized Clinical Trial; CsA: cyclosporine A; MRI: magnetic resonance imaging; OC: Ovarian cysts; CNI: chronic calcineurin inhibitor
IQR: interquartile range; SD: standard deviation

* excluded from the main analysis but included in the sub-analysis for menopausal status (information not present in the updated report)

Three studies were retrospective chart review [8,9,15], ~~one was~~ two were cohort studies [12,13] and two were RCTs [10,14]. Three studies included women with T1DM who underwent allogenic islet transplantation [8,12,13], three kidney transplantation recipients [9,14,15] and one study enrolled women with autosomal dominant polycystic kidney disease [10].

Diagnosis of ovarian cysts was based on pelvic ultrasound examination in four studies [8,9,12,13] with magnetic resonance imaging (MRI) without contrast in one study [10], whereas two did not report the diagnostic criteria [14,15].

SRL was given at increasing dose to reach serum levels ranging from 7 to 15 ng/ml. In one study SRL was given at doses of 1.3 to 1.5 mg SRL per day [11]. TAC target was level 3–6 ng/ ml when given in association with sirolimus [12,13] and or 10 ng/ ml when used in association with mycophenolate mofetil (1 g b.i.d. as tolerated) [8].

Overall, the considered studies included 406 women who received SRL alone or in combination with other drugs, with mean follow-up ranging from 12 to 95 months.

Quality of selected studies

Both Braun et al. [10c] and Gaber et al. [14] had low risk of bias according to the Cochrane risk of bias tool (Table 3).

Table 3. Study quality evaluation according the Newcastle-Ottawa Scale (cohort studies) or Cochrane risk of bias (randomized clinical trials).

Publications							
Cohort study		**Selection**		**Comparability**		**Outcome (CS)**	**Study quality §**
Cure et al, 2004	1 2 3 4	* - * *	1 2	* -	1 2 3	* * -	6/9
Alfadhli et al, 2009	1 2 3 4	* * * -	1 2	* -	1 2 3	* * *	7/9
Del Olmo Garcia et al, 2011	1 2 3 4	* - * *	1 2	- -	1 2 3	* * -	5/9

Ignjatovic et al, 2013	1 2 3 4	* - * *	1 2	- -	1 2 3	- * *	4/9
Bachmann et al, 2018	1 2 3 4	* * * *	1 2	* *	1 2 3	* * *	9/9
RCT							Overall risk of bias
Braun M et al, 2012	Randomization: some concern Assignment to intervention: low risk Adhering to intervention: low risk Missing outcome: low risk Measure of outcome: low risk Selection of results: low risk						Low
Gaber et al, 2008	Randomization: low risk Assignment to intervention: low risk Adhering to intervention: low risk Missing outcome: low risk Measure of outcome: low risk Selection of results: low risk						Low

§ We used the Newcastle– Ottawa quality assessment scale for cohort studies with maximum score 9, as presented at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp (accessed April 24, 2017). Most items were evaluated as "-" because of the small sample size or absence of not exposed cohort.

For the assessment of randomized controlled studies, we used the revised Cochrane risk of bias tool as presented at https://sites.google.com/site/riskofbiastool/welcome/rob-2-0-tool/current-version-of-rob-2

As regards observational cohorts, using the NOS tool study quality was deemed good (8 or 9 out of 9) in Bachmann et al.'s paper [9]. Alfadhli et al.'s study was of some concern because it was unclear if baseline ultrasound scans were detailed enough to identify ovarian cysts [8]. Del Olmo Garcia et al. [13] and Ignjatovic et al. [15] presented mainly descriptive articles, including 18 (13 of whom already included in the paper by Cure et al. [12]) and six women respectively. Therefore, the possibility of some NOS quality item evaluation was debatable (i.e., if sample size was too little to control for important factors or if a not exposed cohort did exist).

Main results

Table 4 reports the frequency of ovarian cysts in women treated with SRL, SRL+TAC and SRL or everolimus. Two studies [8,12] reported the frequency in strata of menopausal status, suggesting that premenopausal women were at higher risk of developing ovarian cysts during mTORi treatment.

Table 4. Results of selected studies: patients with incident ovarian cyst on the total of treated women (%).

	SRL	**SRL + TAC**	**SRL or everolimus**	**All**	**Standard treatment**
Total series					
Gaber et al, 2008	7/98* (7.14)	1/104 (0.96)		8/202 (7.84)	
Alfadhli et al, 2009		33/57 (57.89)		33/57 (57.89)	
Del Olmo Garcia et al, 2011	10/18 (55.56)			10/18 (55.56)	
Ignjatovic et al, 2013	2/6 (33.33)			2/6 (33.33)	
Comparative studies					
Braun M et al 2012	12/21 (57.14)			12/21 (57.14)	5/18 (27.28)
Bachmann et al, 2018			21/102 (20.59)	21/102 (20.59)	23/469 (4.26)
Total	*31/143 (21.68)*	*34/161 (21.12)*	*21/102 (20.59)*	*86/406 (21.18)*	*28/487 (5.75)*
Pre-menopause					
Cure et al, 2004		7/9 (77.78)		7/9 (77.78)	
Alfadhli et al, 2009		31/44 (70.45)		31/44 (70.45)	
Total		*38/53 (71.70)*		*38/53 (71.70)*	

Post-menopause					
Cure et al, 2004		1/4 (25.00)		1/4 (25.00)	
Alfadhli et al, 2009		2/13 (15.38)		2/13 (15.38)	
Total		*3/17 (17.65)*		*3/17 (17.65)*	
Clinically significant					
Alfadhli et al, 2009		14/57 (24.56)			
Del Olmo Garcia et al, 2011	8/18 (44.44)				
Braun M et al 2012	1/21 (4.76)				0/18 (0.00)
Bachmann et al, 2018			10/102 (9.80)		8/487
Total	*9/39 (23.08)*	*14/57* (24.56)	*10/102* (9.80)		*8/505 (1.58)*

*SRL+Cyclosporine

Systematic review

Gaber et al. [14] conducted a RCT to evaluate the efficacy and safety of SRL plus TAC versus SRL plus cyclosporine (CsA) in high-risk renal allograft recipients. A total of 202 women were randomly assigned before transplant to receive SRL-TAC (104 women) or SRL-CsA (98 women) with corticosteroids. Patients randomly assigned to SRL-TAC received a 10-mg loading dose of SRL on days 1 and 2, and 5 mg once daily, thereafter, adjusted to achieve whole blood trough concentrations from 10 to 15 ng/mL (measured by high performance liquid chromatography methodology). Up to 0.2 mg/kg/day of TAC was administered in divided oral doses (twice daily) to achieve whole blood concentrations from 10 to 15 ng/mL between day 1 and week 2, from 5 to 10 ng/mL between weeks 2 and 26, and from 3 to 5 ng/mL between weeks 26 and 52 (measured by monoclonal TDx or equivalent methodology). Patients randomly assigned to SRL-CsA received a larger 15-mg loading dose of SRL on day 1, and 5 mg once daily, thereafter, adjusted to achieve the same whole blood

trough concentrations as the patients assigned to SRL-TAC. One case of ovarian cyst was observed in the SRL-TAC group (1.0%) and seven in the SRL-CsA group (7.1%) (p=0.031). In this study no information on severity of cysts (i.e. for example dimension or presence of pain) was reported.

Alfadhli et al.[8] conducted a chart review retrospective study in 57 women who underwent islet transplantation and received maintenance immunosuppression with SRL (trough levels 12–15 ng/ml for the first 3 months then 7–10 ng/ml thereafter) and TAC (target trough level 3–6 ng/ ml). A small group of patients received TAC at higher doses (target trough levels 10 ng/ml) along with mycophenolate mofetil (1 g b.i.d. as tolerated) for immunosuppression from the time of transplant. Ovarian cysts were found in 33 out of 57 women at a median of 235 (119–405) days after the first islet transplantation: 31 out of 44 (70.5%) premenopausal and two out of 13 (15.4%) postmenopausal women (P = 0.001). Ovarian cysts occurred more frequently in subjects taking SRL plus TAC than those taking high doses of TAC plus mycophenolate mofetil (33/53, 62.3%, vs. 0/4, 0%, P = 0.027). No women using combined oral contraception developed ovarian cysts. Among women taking SRL, average SRL trough levels were similar between those who developed ovarian cysts and those who did not (median 12.1, interquartile range, IQR 10.9–13.3, vs. 12.2, IQR 11.5–12.6 ng/ml, P = 0.993). SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects. The median maximal cyst diameter was 6.0 (3.8–7.6) cm. Most cysts were asymptomatic and noted incidentally on routine imaging. However, 14 subjects (42.4%) reported pelvic pain. In four cases, severe pelvic pain resulted in emergency room visits because of ovarian cyst rupture (n = 2) or torsion (n = 2). Histology was benign in all cases.

Del Olmo Garcia et al. [13] reported a total of 18 subjects (mean age at transplantation 48.5, standard deviation, SD, 8.0 years) with T1DM, who underwent allogeneic transplantation and were treated with SRL, given orally pre-transplant, at 0.2 mg/kg, and then adjusted to achieve trough levels of 12–15ng/ml for the first 90 days and 7–12 ng/ml thereafter. After the transplant, they were followed for a mean time of 7.9 (SD 1.13) years. In this study, a total of ten ovarian cysts (56%) were observed. All the cysts were benign, but eight were considered complex because of haemorrhage, hydrosalpinx,

cyst's size (>6 cm), spontaneous rupture, or need of surgery for resolution. Four women (40%) underwent cystectomy because of poor response to medical treatment. Part of this sample (13 out of 18) was previously described by Cure et al. [12], who reported that in four women, postmenopausal at the time they were transplanted, one case of ovarian cyst was observed, whereas out of nine premenopausal women seven developed ovarian cysts.

Braun et al. [10] reviewed the occurrence of ovarian cysts in a post hoc analysis of an open label randomized controlled phase II trial, conducted between March 2006 and March 2010. Women with autosomal dominant polycystic kidney disease were treated with 1.3 to 1.5 mg SRL per day for a median of 19 months (N = 21) or standard care (N = 18). Ovarian cysts were observed in 12 out of 21 patients in the SRL group, compared to five out of 18 patients in the control group (hazard ratio 4.4, 95% CI 1.1-26). Differences in ovarian cysts between SRL and control did not seem to depend on the contraceptive method (barrier methods: seven out of 11 and three out of nine patients in the SRL and control groups; oral contraceptives: five out of 10 and two out of nine patients in the SRL and control groups). Clinical significance of ovarian cysts was not reported. One patient presented with acute abdominal pain and a large cyst of the left ovary while receiving sirolimus and underwent surgery.

Ignjiatovic et al.[15] reviewed 24 transplant patients (six women) who switched from calcineurin inhibitors (CNI) to SRL from 2003 to 2011. Patients converted from CNI to SRL, with target serum levels 7–10 ng/ml for months 6 to 12 after transplant, and 5-10 ng/ml thereafter. Early after the conversion, two patients developed ovarian cysts with oligomenorrhea and reconverted to CNI, with cyst resolution and return to regular period.

Bachmann et al. [9] compared the effect of mTOR inhibitors vs. non-mTOR inhibitor immunosuppression on the incidence, size and complication rate of ovarian cysts in renal transplant recipients. They retrospectively analyzed 571 consecutive female kidney transplant patients between 2000 and 2008; they were followed-up till December 2012. Of those, 102 (17.8%) patients received mTOR inhibitors for at least one month after transplantation. A total of 44 women (7.7%) with new

ovarian cysts were reported, 21 among patients receiving mTOR inhibitors (20.5%) and 23 in the control group (4.9%). This difference was statistically significant ($p < 0.001$). The hospitalization rate was also more frequent in the mTOR group, with 21 hospitalizations in ten mTORi patients versus nine hospitalizations in eight control subjects ($p = 0.05$). Ten women in the mTOR inhibitor group (9.8%) versus 8 in the control group (1.7%) had symptomatic, clinically significant ovarian cysts requiring surgery.

Synthesis of results

Overall, 406 women were treated with mTORi in the six studies included in this meta-analysis and 86 developed ovarian cysts. The frequency of ovarian cysts in women treated with mTORi, without any specific restriction regarding the type of drug, is reported in Table 5.

Table 5. Pooled estimates of ovarian cyst incidence.

Authors	**Cases**	**Sample size**	**Pooled incidence estimate**	**95% confidence interval**
Gaber et al, 2008	8	202	4.0	2.0-7.6
Alfadhli et al, 2009	33	57	57.9	45.0-69.8
Del Olmo Garcia et al, 2011	10	18	55.6	33.7-75.4
Braun M et al, 2012	12	21	57.1	36.5-75.5
Ignjatovic et al, 2013	2	6	33.3	9.7-70.0
Bachmann et al, 2018	21	102	20.6	13.9-29.4
Random pooled estimate			37.0	16.0-58.1

Heterogeneity χ^2 = 115.0 (d.f. = 5) p = 0.0; I^2 (variation in ES attributable to heterogeneity) = 95.7% Estimate of between-study variance τ^2 = 0.1				
Random pooled estimate excluding Gaber et al.			44.9	23.7-66.1
Heterogeneity χ^2 = 32.5 (d.f. = 4) p = 0.0; I^2 (variation in ES attributable to heterogeneity) = 87.7% Estimate of between-study variance τ^2 = 0.1				

As shown in Table 5, the pooled incidence was 37.0% (95% CI 16.0-58.1) (Figure 2). The rate of ovarian cysts ranged from 4.0% to 57.9%, leading to a remarkable heterogeneity (Chi-square for heterogeneity 115.0, p<0.001, I^2=95.7%). Excluding the study with most extreme results [14] the pooled estimate increased to 44.9% (95% CI 23.7-66.1), with a small decrease of heterogeneity, that remained, however, remarkable.

As shown in Table 4, pooling the results of two comparative studies [8,12], we found that ovarian cyst rates were higher for premenopausal women (38/53, 71.7%) than postmenopausal ones (3/17, 17.6%) and the difference was statistically significant (p<0.0001): the odds ratio for developing ovarian cysts was 12.46 (95% CI 3.04-50.98) comparing pre-menopausal with post-menopausal women.

Two studies compared mTOR inhibitors vs. non-mTOR inhibitor immunosuppression [9,10]. The pooled odds ratio for ovarian cyst incidence was 4.62 (95% CI 2.58-8.28) and the pooled odds ratio for clinically significant ovarian cysts was 5.56 (95% CI 2.34-14.67).

Finally, we pooled the incidence of clinically significant ovarian cysts in studies reporting this information [8–10,12]. The resulting estimate was 17.3% (95% CI 5.6-29.1), heterogeneity chi-square 15.5, p<0.001) (Figure 3).

Discussion.

This systematic review shows that, in women treated with mTOR inhibitors, the incidences of ovarian cysts ranged between less than 10% to more than 50%, in different studies and clinical series. The pooled incidence was 37%, 17% only considering clinically significant ovarian cysts. The risk seems to be higher among premenopausal women: two studies distinguished ovarian cyst incidence occurring in pre- and post-menopausal patients, with consistent results [8,12], suggesting that mTORi effect is higher in presence of spontaneous ovarian activity.

Where immunosuppression was achieved using mTOR inhibitors as compared to non-mTOR inhibitors [9,10], women on mTOR inhibitors were at higher risk of developing ovarian cysts.

The limited data and the differences in the presentation of results do not provide the opportunity of analyzing in detail the role of stopping mTORi on the clinical course of ovarian cysts or the protective role of oral contraceptive use.

In the study by Alfadhli et al. [8], SRL withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of subjects; however, the proportion with partial or complete cyst resolution was similar in those who did or did not discontinue SRL (12/15, 80%, vs. 6/8, 75%, P = NS).

Another potential risk factor for the development of ovarian cysts during mTORi use was a previous history of ovarian cysts; Del Olmo Garcia et al. [13] reported five patients with such a history.

In our synthesis, we found an extremely high heterogeneity, that may be due to both the different criteria for diagnosis of ovarian cysts and the type of disease requiring mTORi use. For example, T1DM is associated with menstrual irregularity and PCOS [17,18], hence a higher basal frequency of ovarian cyst development. Another factor likely affecting heterogeneity was the active screening for ovarian cysts development in women on mTORi treatment. In particular, it appears that in the study with the lowest incidence [14], women did not routinely undergo abdominal scans: pelvic (i.e., transvaginal) sonography would be the preferred imaging modality to exclude ovarian cysts.

In biological terms, mTOR inhibitors may affect the levels of LH and FSH. Further, expression of progesterone receptors can be inhibited by sirolimus via the mTOR and inhibition of progesterone

receptors in the ovaries may interfere with ovarian cysts development. However, the specific mechanisms linking mTOR inhibitor exposure and risk of developing ovarian cysts are unknown [12].

This review and meta-analysis may be affected by potential limitations or bias.

Findings from this systematic review and meta-analysis are based on an extremely limited number of studies, thus the results should be considered cautiously. Taking this aspect into account, the general results confirm clinical suggestion that mTORi increase the frequency of benign ovarian cysts.

Among studies, the heterogeneity was remarkable. This finding may be due to several characteristics of the selected samples. First, two studies included women with T1Dm who underwent allogeneic islet transplantation [8,13], three included women who underwent renal transplantation [9,14,15] and one study women with autosomal dominant polycystic kidney [10]. Then, the ascertainment of ovarian cysts was performed with different methodologies, variable imaging modalities and definitions (size, persistence) of ovarian cyst. Whereas certain studies defined ovarian cysts as cystic formation >2cm in MRI images [11], other studies included only cysts of >3cm not resolving spontaneously after 4 months diagnosed by transvaginal sonography [12,13] and in two studies the method was not reported [14,15]. Thus, in order to reduce the heterogeneity in the definition of ovarian cysts among the considered studies we have also performed a meta-analysis of clinically significant ovarian cysts. Lastly, the number of study participants was quite different among studies ranging between 6 and more than 200 women. Despite this, the pooled estimate is not overwhelmingly affected by the largest studies [9,14], and the study weights are similar (Figure 2).

We considered only publications published in English. Authors may be more prone to publish in an international, English-language journal if results are positive, whereas negative findings are more often published in local journals [19]. Limiting our analysis to publications in English language journals can therefore restrict the completeness of information, thereby causing bias. The direction and the strength of this bias are not however clear.

Another limitation is the fact that most of studies included an extremely limited number of subjects. Although systematic reviews with meta-analyses provide an explicit method for synthesizing evidence and overcome the low power of the single studies, they may not be as valuable as a single large observational study. Lastly, this study was not registered a priori.

Despite these limitations, consistent results among all studies give strong support to the general findings.

Although the biological and clinical explanation of the results of our analysis is not totally clear, observational studies and clinical trials consistently suggest that ovarian cysts are a common adverse effect of mTOR inhibitors. These cysts are benign conditions, but they require pelvic ultrasound follow-up and in some cases hospital admission and surgery. Based on these considerations, women and physicians should be warned in routine clinical practice about the gynecological impact of long-term use of mTOR inhibitors. Further the risk of ovarian cyst, together with the impact of mTOR inhibitors on glucose metabolism, risk of diabetes and other potential adverse effects should be included in the risk benefit balance of mTOR Inhibitors use as immunosuppressive agents.

Conflict of interest: all authors declare no conflict of interest.

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Authors' contribution

Stefano Bianchi, Sergio Harari, Fabio Parazzini and Sandro Gerli designed the study; Andrea Dell'Acqua, Alessandro Favilli and Michele Vignali reviewed the text; Fabio Parazzini, Elena Ricci and Francesca Chiaffarino performed the literature research and extracted the data; Sonia Cipriani and Elena Ricci performed the statistical analyses; Stefano Bianchi and Fabio Parazzini wrote the paper.

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information

Ethics Statement

This study did not involve human participants

References

[1] Zheng Y, Jiang Y. mTOR Inhibitors at a Glance. Mol Cell Pharmacol 2015;7:15–20.

[2] Alam H, Maizels E, Park Y, Ghaey S, Feiger Z, Chandel N, et al. Follicle-stimulating hormone activation of hypoxia-inducible factor-1 by the phosphatidylinositol 3-kinase/AKT/Ras homolog enriched in brain (Rheb)/ mammalian target of rapamycin (mTOR) pathway is necessary for induction of select protein markers of follicular differentiation. J Biol Chem 2004;279:19431–40.

[3] Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med 2009;6:e1000097. https://doi.org/10.1371/journal.pmed.1000097.

[4] Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of non randomised studies in meta-analyses n.d. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

[5] Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. Br Med J 2019;366:1–8. https://doi.org/10.1136/bmj.l4898.

[6] Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. Arch Public Heal 2014;72:39.

[7] Greenland S. Quantitative methods in the review of epidemiologic literature. Epidemiol Rev 1987;9:1–30.

[8] Alfadhli E, Koh A, Albaker W, Bhargava R, Ackerman T, McDonald C, et al. High prevalence of ovarian cysts in premenopausal women receiving sirolimus and tacrolimus after clinical islet transplantation. Transpl Int 2009;22:622–5. https://doi.org/10.1111/j.1432-2277.2009.00839.x.

[9] Bachmann F, Glander P, Budde K, Bachmann C. High Incidence of Ovarian Cysts in Women Receiving mTOR Inhibitors after Renal Transplantation. J Women's Heal

2018;27:394–8. https://doi.org/10.1089/jwh.2017.6451.

[10] Braun M, Young J, Reiner CS, Poster D, Krauer F, Kistler AD, et al. Low-Dose Oral Sirolimus and the Risk of Menstrual-Cycle Disturbances and Ovarian Cysts: Analysis of the Randomized Controlled SUISSE ADPKD Trial. PLoS One 2012;7:e45868. https://doi.org/10.1371/journal.pone.0045868.

[11] Braun M, Young J, Reiner C, Poster D, Wüthrich R, Serra A. Ovarian toxicity from sirolimus. N Engl J Med 2012;366:1062–4.

[12] Cure P, Pileggi A, Froud T, Norris PM, Baidal DA, Cornejo A, et al. Alterations of the female reproductive system in recipients of islet grafts. Transplantation 2004;78:1576–81. https://doi.org/10.1097/01.TP.0000147301.41864.C0.

[13] Garcia MIDO, Lauriola V, Aracena AG, Messinger S, Corrales A, Ricordi C, et al. Alterations of the female reproductive system in islet recipient receiving immunosuppression. Cell Transplant 2011;20:1649–51. https://doi.org/10.3727/096368910x557209.

[14] Gaber AO, Kahan BD, Van Buren C, Schulman SL, Scarola J, Neylan JF. Comparison of sirolimus plus tacrolimus versus sirolimus plus cyclosporine in high-risk renal allograft recipients: Results from an open-label, randomized Trial. Transplantation 2008;86:1187–95. https://doi.org/10.1097/TP.0b013e318187bab0.

[15] Ignjatović L, Hrvacević R, Jovanović D, Kovacević Z, Vavić N, Rabrenović V, et al. Conversion from calcineurin inhibitors to sirolimus of recipients with chronic kidney graft disease grade III for a period 2003-2011. Vojn Pregl 2013;70:848–53.

[16] Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, et al. Five-Year Follow-Up After Clinical Islet Transplantation. Diabetes 2005;54:2060–9.

[17] Strotmeyer ES, Steenkiste AR, Foley TP, Berga SL, Dorman JS. Menstrual cycle differences between women with type 1 diabetes and women without diabetes. Diabetes Care 2003;26:1016–21. https://doi.org/10.2337/diacare.26.4.1016.

[18] Escobar-Morreale HF, Roldán B, Barrio R, Alonso M, Sancho J, De La Calle H, et al. High prevalence of the polycystic ovary syndrome and hirsutism in women with type 1 diabetes mellitus. J Clin Endocrinol Metab 2000;85:4182–7. https://doi.org/10.1210/jc.85.11.4182.

[19] Grégoire G, Derderian F, Le Lorier J. Selecting the language of the publications included in a meta-analysis: is there a Tower of Babel bias? J Clin Epidemiol 1995;48:159–63.

Figure 1. Flow chart of selected studies.

Figure 2. Forest plot of ovarian cyst incidence.

Figure 3. Forest plot of clinically significant ovarian cyst incidence.

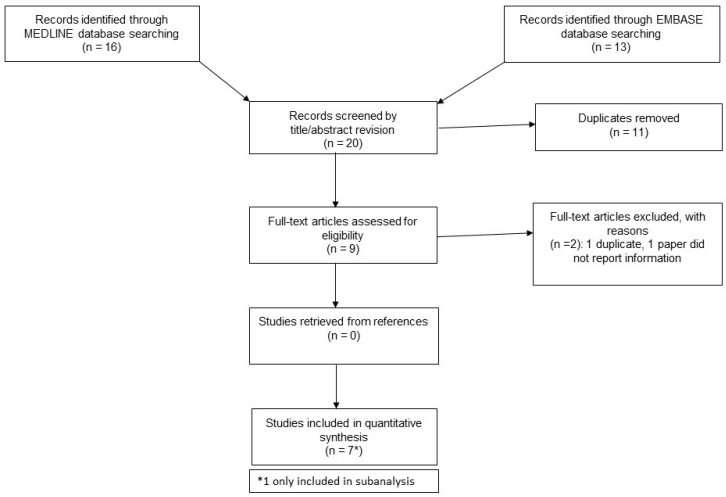

Figure 1. Flow chart of selected studies.

338x190mm (96 x 96 DPI)

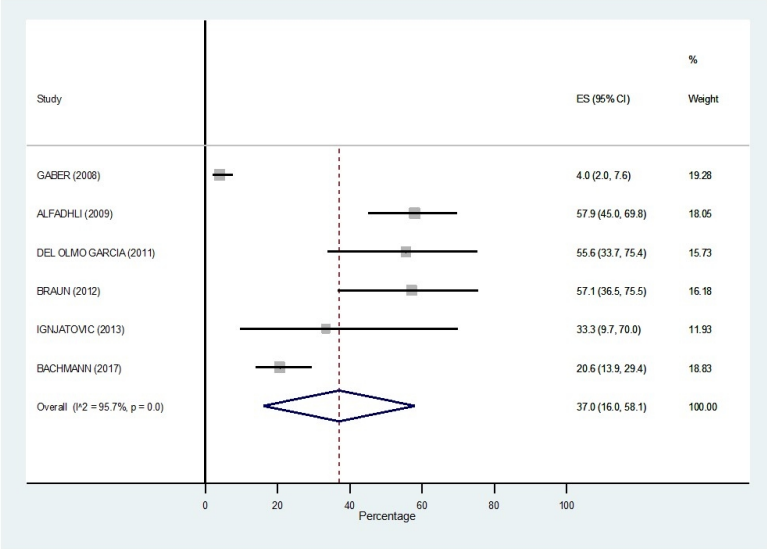

Figure 2. Forest plot of ovarian cyst incidence.

338x190mm (96 x 96 DPI)

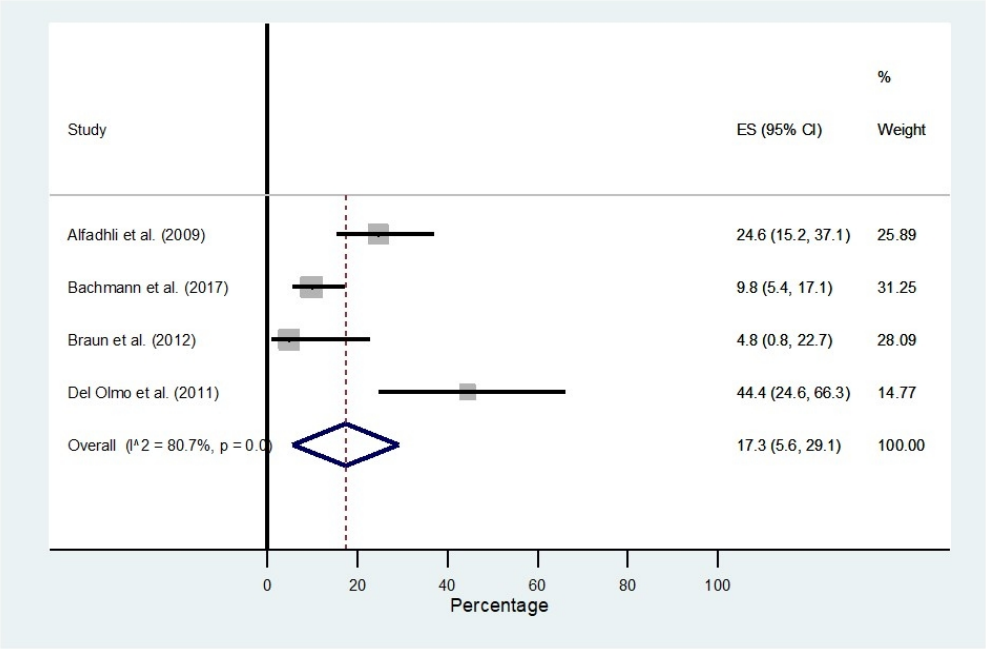

Figure 3. Forest plot of clinically significant ovarian cyst incidence.

251x166mm (96 x 96 DPI)

Search Strategy

PubMed/Medline

(("tacrolimus"[MeSH Terms] OR "tacrolimus"[All Fields] OR ("sirolimus"[MeSH Terms] OR "sirolimus"[All Fields]) OR ("everolimus"[MeSH Terms] OR "everolimus"[All Fields]) OR ("ridaforolimus"[Supplementary Concept] OR "ridaforolimus"[All Fields] OR "deforolimus"[All Fields]) OR "mTOR"[All Fields]) AND ("ovarian cysts"[All Fields] OR "ovarian cyst"[All Fields])) AND ((humans[Filter]) AND (english[Filter])) AND (("1990/01/01"[PDAT]: "2020/03/31"[PDAT])

EMBASE

('tacrolimus'/exp OR tacrolimus OR 'sirolimus'/exp OR sirolimus OR 'everolimus'/exp OR everolimus OR 'deforolimus'/exp OR deforolimus OR 'mtor'/exp OR mtor) AND ('ovarian cysts'/exp OR 'ovarian cysts' OR 'ovarian cyst'/exp OR 'ovarian cyst') AND [article]/lim AND [humans]/lim AND [english]/lim AND [embase]/lim AND (1990:py OR1991:py OR 1992:py OR 1993:py OR 1994:py OR 1995:py OR 1996:py OR 1997:py OR 1998:py OR 1999:py OR 2000:py OR 2001:py OR 2002:py OR 2003:py OR 2004:py OR2005:py OR 2006:py OR 2007:py OR 2008:py OR 2009:py OR 2010:py OR 2011:py OR 2012:py OR 2013:py OR 2014:py OR 2015:py OR 2016:py OR 2017:py OR 2018:py OR 2019:py OR 2020:py)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6 Table 1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	-
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5-6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5-6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	-
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6 Table 1
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5-6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	6-7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6-7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6-7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	13-16 Table 4 Figure 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	17-18 Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	-
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	19-20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	20
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	21

PRISMA 2009 Checklist

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097