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Tobacco smoking and risk of endometriosis: a systematic review and meta-analysis

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Abbreviations: CI: confidence interval, HR: hazard ratio, MOOSE: meta-analysis of observational studies in epidemiology, OR: odds ratio, RR: relative risk.

ABSTRACT

Objective: Since conflicting results have been published on the role of tobacco smoking on the risk of endometriosis, we provide an up to date summary quantification of this potential association.

Design: We performed a PubMed/MEDLINE search of the relevant publications up to May 2012, considering studies on humans published in English. We searched the reference list of the identified papers to identify other relevant publications. Both case-control or cohort studies have been included reporting risk estimates on the association between tobacco smoking and endometriosis. Thirty-three out of the 1,534 screened papers met the inclusion criteria. The selected studies included a total of 8,225 women diagnosed with endometriosis.

Setting: Academic hospitals

Main outcome measures: Risk of endometriosis in tobacco smokers.

Results: We obtained the summary estimates of the relative risk (RR) using the random-effect model, and assessed the heterogeneity among studies using the χ^2 test and quantified it using the I^2 statistic. As compared to never smokers, the summary RR were 0.97 (95% confidence interval, CI: 0.86-1.09) for ever smokers, 0.95 (95% CI: 0.81-1.11) for former smokers, 0.94 (95% CI: 0.83-1.06) for current smokers, 0.87 (95% CI: 0.70-1.07) for moderate smokers, and 0.93 (95% CI: 0.69-1.26) for heavy smokers.

Conclusions: The present meta-analysis provided no evidence for an association between tobacco smoking and the risk of endometriosis. The results were consistent considering

ever, former, current, moderate, and heavy smokers, and across type of endometriosis and study design.

Strengths and limitations of the study

- Meta-analysis including 33 papers without any relevant asymmetry in the funnel plot.
- The Egger's test was not statistically significant.
- In some studies, choice of the cases as symptomatic without distinguishing factors related to endometriosis to those associated to pelvic pain or infertility.
- In some studies, choice of controls in whom disease was not laparoscopically ruled out.
- Tobacco smoking based on patients' self-reported information.

INTRODUCTION

Endometriosis is an estrogen-dependent, chronic inflammatory gynecological condition characterized by the proliferation of functional endometrial tissue that develops outside the uterine cavity, which may cause pain and infertility [1]. However, despite its relatively high prevalence, which spans from 20% in asymptomatic women [2], to 30% in women with infertility [3], and 45% in women with pain symptoms [4], risk factors for this condition remain largely unknown.

Among the risk factors investigated, some studies have examined the role of tobacco smoking. In a Portuguese study investigating clinical and lifestyle factors in infertile women, current smokers had a decreased risk of endometriosis as compared to non-smokers or former smokers [5]. In a case-control study from Turkey evaluating the interaction between tobacco smoking and glutathione-S-transferase gene polymorphism as a risk factor for endometriosis, an inverse association between smoking and endometriosis was observed [6]. In a case-control study carried out in the USA, infertile women with endometriosis and fertile controls were compared and a decreased risk of endometriosis was found, though limited to women who begun smoking at an early age and were heavy smokers [7]. Other studies did not find significant association [3, 8-14].

The biological plausibility potentially linking smoking and endometriosis resides in its endocrine and inflammatory mechanisms. Smoke compounds disrupt steroidogenesis, leading to impairment of E2 synthesis [15, 16] and progesterone synthesis deficiency [17-19]. Moreover, smoking has a strong effect on inflammatory mediators in both the pulmonary

and extra-pulmonary environments and can further trigger inflammation associated with the disease resulting in pro-inflammatory gene overexpression [20].

Thus, in order to investigate the possible relation between tobacco smoking and endometriosis, and to provide an overall quantitative estimate of any such relation, we combined in a meta-analysis all published data on the issue.

MATERIALS AND METHODS

Search strategy

We performed a PubMed/MEDLINE search of papers published between 1966 and May 2012, using the terms "tobacco" or "smoking" or "cigarette" in combination with "risk factor", or "epidemiology", and "endometriosis", following the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines [21]. We selected only studies on humans, published as full-length papers in English. No effort was made to identify papers published in other languages or unpublished studies. Moreover, we reviewed the reference lists of the retrieved papers, to identify any other relevant publication. Studies were included in the meta-analysis if: a) they were based on case-control or cohort studies, reporting original data; b) they reported information on the association between tobacco smoking and endometriosis, including estimates of the relative risk (RR) or the odds ratio (OR) or the hazard ratio (HR), with the corresponding 95% confidence intervals (CI), or frequency distribution to calculate them; c) diagnosis of endometriosis was histologically confirmed and/or clinically based. When we found more than one publication based on the same study

population and data, we included only the one with most detailed information, or published most recently.

Data extraction for the meta-analysis

From each publication we extracted the following information: country of origin; study design; number and characteristics of subjects (cases, controls or cohort size); age, if available; categories of tobacco smoking, if available; measures of association (RR, or OR or HR) of endometriosis and corresponding 95% CI for every category of tobacco smoking, or frequency distribution to calculate them; confounding variables allowed for in the statistical analysis, if any. When more than one regression model was provided, estimates adjusted for the largest number of confounding variables were considered.

Statistical analysis

For some studies, we pooled estimates of different categories of cases or controls using the method by Hamling et al. [22], thus taking into account their correlation. We obtained the summary estimates of the RR using the random-effect model (i.e., as weighed averages on the sum of the inverse of the variance of the log RR and the moment estimator of the variance between studies) [23]. We assessed the heterogeneity among studies using the χ^2 test [24] and quantified it using the I^2 statistic, which represents the percentage of the total variation across studies that is attributable to heterogeneity rather than chance[25]. Results were defined as heterogeneous for P values less than 0.10.

We computed summary estimates for ever tobacco smokers, former smokers, current smokers, moderate current smokers, and heavy current smokers, as compared to never smokers. Different cut-points for moderate and heavy smoking were chosen, depending on

those shown in the papers. We also carried out a cumulative meta-analysis to determine whether the association between tobacco smoking and endometriosis changed over time and performed subgroup analyses according to type of controls (fertile, infertile, both/not specified). Publication bias was evaluated using funnel plot [26] and was quantified by the Egger's test [27].

RESULTS

Figure 1 shows the flow-chart of the selection of publications. From the literature search we identified 1534 studies, 1448 of which were excluded because not relevant, and 40 because did not satisfy the inclusion criteria. Moreover, 3 studies were not comparable with the other ones, since reported estimates for lifetime smoking [2], included former or light smokers in the reference category [1], or included women with stage I endometriosis in the comparison group, and thus we excluded those studies from the meta-analysis. Furthermore, we excluded 14 studies based on the same data of other included publications [28-42]. Thus, in the present meta-analysis we combined data from 33 studies, including a total of 8225 women with endometriosis (suppl. file, Table 1) [3, 5-10, 12-14, 43-65].

Table 1 shows the main characteristics of the studies included in the present meta-analysis. Most publications were based on case-control studies, while six were cohort studies, in which, however, the role of smoking was not evaluated prospectively [13, 43, 45, 47, 50], except in one case [5]. Of these, 14 studies were from Europe [3, 5, 9, 10, 45, 47-49, 52, 54, 58, 60-62], 12 from the USA [7, 12-14, 43, 46, 50, 53, 56, 57, 59, 63], 2 from Canada [8, 55], 4 from Asia [6, 44, 51, 65], and 1 from Australia [6].

Twenty-one studies reported information on ever smokers [5, 7-10, 13, 14, 43, 45, 47-49, 52, 53, 56, 59, 60, 62-65], 16 on former smokers [5, 7-10, 13, 45, 47-49, 53, 56, 60, 62-64], and 28 on current smokers [3, 5-10, 12, 13, 44-51, 53-58, 60-64]. Among these, 8 reported more categories of current smokers, thus we could calculate separate estimates for moderate and heavy current smokers. We used different cut-points for various study populations, depending on those presented in the papers: thus the cut-point between moderate and heavy smokers were defined as 20 cigarettes per day in five studies [5, 8, 46, 62, 63], 15 cigarettes per day in two studies [13, 50] and 10 cigarettes per day in one study [10].

For some studies reporting separate estimates for different types of patients and/or controls, we computed a pooled estimate. In particular, Coccia et al. [45] reported separate estimates for monolateral and bilateral endometriosis, Heilier et al. [49] for endometriosis and deep endometriotic nodules, Parazzini et al. [60] for deep endometriosis and pelvic and ovarian endometriosis, Signorello et al. [14] for fertile and infertile controls, Tsuchiya et al. [65] for stage I/II and stage III/IV endometriosis. Moreover, Calahz-Jorge et al. [5] reported separate estimates for grade I/II and grade III/IV endometriosis, as well as for any type of endometriosis, and the Gruppo Italiano per lo Studio dell'endometriosi [10], including two separate groups of cases and controls undergoing laparoscopy for pelvic pain or infertility, showed both separate and pooled estimate; in both cases we included in the meta-analysis the combined estimates.

Figure 2 shows the study-specific and summary RRs of endometriosis for ever smokers versus non smokers. The summary RR from 21 studies was 0.97 (95% CI: 0.86-1.09)(x^2 heterogeneity between studies =37.23, p=0.011). Figure 3 gives the study-specific and

summary RR of current (A) and former (B) smokers versus never smokers. The summary RR of current versus never smokers was 0.94 (95% CI: 0.83-1.06) from 28 studies (x^2 heterogeneity =54.76, p=0.001). The summary RR of former versus never smokers was 0.95 (95% CI: 0.81-1.11) from 16 studies, with hetergogeneity (x^2=30.63, p=0.010). Figure 4 shows the RR of moderate (A) and heavy (B) current smokers versus non smokers, respectively. The summary RR from 8 studies were 0.87 (95% CI: 0.70-1.07)(x^2 heterogeneity =12.58, p=0.083), and 0.93 (95% CI: 0.69-1.26)(x^2 heterogeneity =17.21, p=0.016), for moderate and heavy smokers, respectively.

Figure 5 shows the funnel plot for ever smokers versus non smokers. There was no evidence of publication bias (p=0.924).

When we restricted the analyses to 8 studies reporting risk estimates adjusted for confounding variables, risk estimates were 0.90 (95% CI: 0.77-1.06) for ever smokers, 0.87 (95% CI: 0.75-1.01) for former smokers, 0.86 (95% CI: 0.71-1.06) for current smokers, 0.87 (95% CI: 0.65-1.15) for moderate current smokers, and 0.95 (95% CI: 0.66-1.37) for heavy current smokers versus never smokers.

In subgroup analyses according to type of controls, estimates for ever versus non smokers were 0.97 (95% CI: 0.81-1.17) for 7 studies including fertile women, 0.92 (95% CI: 0.75-1.12) for 6 studies including infertile women, and 0.99 (95% CI: 0.83-1.19) for 12 studies including both or not specified type of controls. Moreover, when we restricted the analyses to studies with cases and controls laparoscopically or surgically confirmed, the risk estimates were 0.98 (95% CI:0.87-1.09) for ever smokers, 0.94 (95% CI: 0.85-1.03) for

former smokers, 0.91 (95 % CI: 0.77-1.07) for current smokers, 0.86 (95% CI: 0.66-1.12) for moderate smokers, and 0.97 (95% CI: 0.70-1.35) for heavy smokers.

Figure 6 shows the cumulative meta-analysis of endometriosis risk for ever smokers versus non smokers over time, from 1986 to 2011. The estimate was 0.90 (95% CI: 0.70-1.15) in 1986 and 0.97 (95% CI: 0.86-1.09), with a few small variations over time, all the estimates being not significantly below unity.

DISCUSSION

The present meta-analysis do not support an association between smoking and endometriosis risk. No association emerged considering subgroups of ever, former, current, moderate and heavy smokers.

This work may be affected by limitations and biases intrinsic in the observational studies included in the meta-analysis. A major concern is the choice of the comparison group. Some studies compared symptomatic cases with asymptomatic controls, and thus could not distinguish factors related to endometriosis to those associated to pelvic pain or infertility. Moreover, generally asymptomatic controls did not undergo laparoscopy nor other surgical procedures, and therefore the presence of asymptomatic endometriosis in these women cannot be ruled out. However, when we restricted the analyses to women in whom laparoscopy or a surgical procedure had confirmed the presence or absence of endometriotic lesions, still we did not find any significant association between smoking and endometriosis of concern is the fact that in some studies diagnosis of endometriosis was self reported. Further, tobacco smoking is based on patients' self-reported information, thus

some misclassification may have occurred. However, information on tobacco smoking in observational studies has been shown to be satisfactorily reproducible and valid [66-68]. Fourth, for most studies included in the present meta-analysis only raw estimates were available, since tobacco smoking was not the main topic of the paper and it was only reported as confounding variable. However, estimates from these studies were similar to those from studies specifically investigating the role of smoking, thus, allowing to rule out major publication bias on this issue. Moreover, we did not find any relevant asymmetry in the funnel plot, and the Egger's test was not statistically significant. Thus, publication bias is unlikely to have appreciably modified the relation between tobacco smoking and endometriosis. Fifth, although previous studies have reported an association between endometriosis and menstrual and reproductive factors, such as early menarche [7, 12], longer duration of bleeding [7], intra-uterine device use [69], or a lifelong regular menstrual pattern of shorter cycles and heavy flows [7, 12, 63, 70], nulliparity or low parity [14, 28, 33, 71], only some studies included in the present meta-analysis have accounted for the role of these factors in the estimate of the relation between tobacco smoking and endometriosis. However, analyses based on adjusted estimates only were comparable to those based on raw estimates.

Since endometriosis is an estrogen-dependent condition, the inverse association between smoking and endometriosis found in some studies has generally been attributed to the antiestrogenic effect of tobacco [72]. Some authors have suggested that estradiol might modulate the mediators of immune system molecules or those involved in tissue cell adhesion and invasion [73, 74]. Moreover, a favorable effect of smoking has been observed in

other benign and malignant estrogen-related diseases, such as endometrial cancer [75], and fibroids [76]. The antiestrogenic effect of smoking on these conditions could support a protective effect of smoking on endometriosis. Indeed, earlier studies tended to support some inverse association, which however declined over time, and accumulating evidence suggests the presence of some false positive findings in earlier studies [77]. Furthermore, tobacco smoking has been associated with female infertility [78], and thus the interpretation of the relation between smoking and endometriosis may be influenced by the role of infertility.

Despite the high prevalence of this condition, the epidemiology of endometriosis still needs to be elucidated, for several reasons. Endometriosis is a complex condition in which a genetic contribution and environmental factors seem to be involved [79]. Further, it is a disease characterized by a still poorly defined phenotype. The disease stage depends on the type (cysts, implants, nodules), location (ovary, peritoneum, bladder, ureter, etc.), appearance and depth of invasion of the lesions, that can vary greatly among patients. The clinical presentation can be so variable and the lesions of such diverse morphology that none of the pathogenetic models proposed (retrograde menstruation, coelomic metaplasia, embryological origin) can fully explain the various aspects of endometriosis, and none has been recognized as an ultimately valid explanatory model for all the different forms and manifestations of the disease [79]. Moreover, an invasive procedure is needed to diagnose it [79, 80]. Furthermore, published studies differ in the case and control selection and population definition, depending on the choices to consider fertile or infertile cases, and healthy controls or patients with conditions other than endometriosis. Despite these possible

sources of variations, the consistency of results observed weighs against any relevant role of tobacco on endometriosis.

In conclusion, the present meta-analysis gives no support to the hypothesis of an association between tobacco smoking and endometriosis. However further studies are needed to evaluate in deep the time out relationship and the potential effect of smoking a different type of endometriosis.

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Contributors

F.P. conceived the idea and planned the research. FB and SC performed the statistical analysis. FB, FC, ER, VC retrieved data. FP, FB, PV and CLV wrote the entire draft of the article and all subsequent drafts after critical review by all co-authors. All co-author had significant input in the preparation of the article and the analysis. FP is the guarantor for the article.

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FIGURE LEGENDS

Figure 1 – Flow chart of the selection of studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

Figure 2 – Study-specific and summary relative risks (RR) of endometriosis for ever smokers versus non smokers.

CI: confidence interval.

Figure 3 – Study-specific and summary relative risks (RR) of endometriosis for current (A) and former smokers (B) versus non smokers.

CI: confidence interval.

Figure 4 – Study-specific and summary relative risks (RR) of endometriosis for moderate (A) and heavy (B) current smokers versus non smokers.

CI: confidence interval.

Figure 5 – Funnel-plot of studies on tobacco smoking and risk of endometriosis.

RR: relative risk for ever smokers versus non smokers; CI: confidence interval; s.e.: standard error.

Figure 6 - Cumulative meta-analysis of studies on tobacco smoking and risk of endometriosis.

RR: relative risk for coffee consumption versus no consumption; CI: confidence interval.

Supplementary file

Table 1 – Main characteristics of the studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Aban et al., 2007 [6]	Turkey	Case-control	Women with endometriosis (surgically and histologically confirmed)	Women without endometriosis (surgically confirmed) undergoing tubal ligation, infertility workup, or ovarian cystis workup	150/150	mean 33.06 ± 8.67 for cases and 34.04 ± 9.68 for controls	Never, current smoker	Body mass index, age at menarche, education, socioeconomic status, cycle length, duration of bleeding
Berubé et al., 1998 [8]	Canada	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	329/262	20-39	Never, former, current smoker (<20, ≥20 cigarettes/day)	-
Buck Louis et al., 2007 [43]	USA	Cohort	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis	32/52	18-40	Never, ever smoker	Age
Calhaz-Jorge et al., 2004 [5]	Portugal	Cohort	Infertile women with endometriosis (laparoscopically confirmed); separate groups of grade I-II and grade III/IV endometriosis	Infertile women without endometriosis (laparoscopically confirmed)	488/591	mean 30.9 ± 3.9 for AFS grade I/II, 30.7 ± 4.0 for ASF grade III/IV and 30.9 ± 4.2 for controls	Never, former, current smoker (1-10, 11-20, >20 cigarettes/day)	Ethnicity, dysmenorrhoea, chronic pelvic pain, cycle regularity, body mass index, previous pregnancies, ever OC use
Cayan et al., 2010 [44]	Turkey	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	135/135	mean 39.36 ± 8.88 for cases and 41.6 ± 8.92 for controls	Non smoker, smoker	-
Chapron et al., 2010 [9]	France	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	411/567	<42 years	Ever, former, current smoker	Age, ethnicity, gravidity, parity, infertility, body mass index

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Coccia et al., 2011 [45]	Italy	Cohort	Women with endometriosis (laparoscopically confirmed) Separate groups of monolateral and bilateral endometriosis	Women without endometriosis (laparoscopically confirmed)	239/63	mean 32.6 ± 5.6	Never, former, current smoker	-
Cramer et al., 1986 [7]	USA	Case-control	Infertile women with endometriosis	Women admitted to hospital for delivery	268/3794	NA	Never, former, current smoker	Center, age, education, religion, years since menarche, menstrual pain, cycle length, weight, height, exercise
Dhillon et al., 2003 [46]	USA	Case-control	Women with cystic ovarian endometriosis (endometrioma)	Women receiving care from the same health maintenance organization	77/735	18-39	Non smoker, smoker (≤0.5, 0.5-1, ≥1 packs/day)	-
Eskenazi et al., 2002 [47]	Italy	Cohort	Women ≤30 yrs in 1976 with stored sera resident near Seveso in1976, with endometriosis (confirmed through laparoscopy, laparotomy or ultrasound)	Women ≤30 yrs in 1976 with stored sera resident near Seveso in 1976	19/277	≥20	Never, former, current smoker	-
Ferrero et al., 2005 [48]	Italy	Case-control	Women of reproductive age undergoing surgery because of uterine myomas, ovarian cysts, pelvic pain, dysmenhorrea, or infertility with endometriosis (histologically confirmed)	Women of reproductive age undergoing surgery because of uterine myomas, ovarian cysts, pelvic pain, dysmenhorrea, or infertility without endometriosis (histologically confirmed)	467/412	mean 34.3 ± 6.0 for cases and 34.5 ± 4.9 for controls	Never, former, current smoker	-

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Gruppo Italiano per lo Studio dell'endometriosi, 1999 [10]	Italy	Case-control	Women with infertility or pelvic pain with endometriosis (laparoscopically confirmed); separate groups of pelvic pain and infertility	Women with infertility or pelvic pain without endometriosis (laparoscopically confirmed); separate groups of pelvic pain and infertility	345/472	18-43	Never, former, current smoker (<10, ≥10 cigarettes/day)	Age, parity, center, education, marital status
Heilier et al., 2007 [49]	Belgium	Case-control	Women with peritoneal endometriosis or deep endometriotic nodules (surgically confirmed); separate groups of endometriosis and deep endometriotic nodules	Women who consulted the same gynecologists of cases, with no clinical evidence of endometriosis	88+88/88	21-50	Never, former, current smoker	-
Hoffman et al., 2007 [50]	USA	Cohort	Women enrolled in the Michigan Polybrominated Biphenyls cohort, with self-reported endometriosis	Women enrolled in the Michigan Polybrominated Biphenyls cohort, without endometriosis	79/864	mean 45 ± 14.4	Non, current smoker (1-15, >15 cigarettes/day)	.
Huang al., 2010 [51]	Taiwan	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis, adenomyosis and leiomyomas (laparoscopically confirmed)	28/29	mean 34.3 ±7.5 for cases and 36.2 ± 9.0 for controls	Current smoker	-
Huber et al., 2005 [52]	Austria	Case-control	Women with endometriosis (surgically and histologically confirmed)	Healthy women without endometriosis (based on personal interview)	32/790	mean 52.3 ± 5.4 for cases and 34.6 ±7.0 for controls	Ever smoker	-
Jackson et al., 2008 [53]	USA (NHANES study)	Case-control	Women with self-reported diagnosis of endometriosis	Women without self-reported diagnosis of endometriosis	61/1362	20-49	Never, former, current smoker	-

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Kortelahti et al., 2003 [54]	Finland	Case-control	Women with endometriosis (histologically confirmed)	Women who underwent laparoscopy for tubal sterilization, and women who underwent in vitro fertilization for reasons other than endometriosis	137/137	mean 31.2 ± 5.1 for cases and 34.0 ± 4.6 for controls	Current smoker	-
Lebel et al., 1998 [55]	Canada	Case-control	Premenopausal women with endometriosis (laparoscopically confirmed)	Premenopausal women without endometriosis (laparoscopically confirmed)	86/70	18-50	Current non smoker	-
Marino et al., 2009 [56]	USA	Case-control	Women enrolled in a health maintenance organization with surgically confirmed endometriosis	Women enrolled in a health maintenance organization without endometriosis	313/727	18-49	Never, former, current smoker	-
Matalliotakis et al., 2008 [12]	USA	Case-control	Women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis undergoing laparoscopy	535/200	15-56	Current smoker	-
Matorras et al., 1995 [3]	Spain	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	174/174	mean 29.49 ± 3.41 for cases and 29.58 ± 3.66 for controls	Current smoker	-
McCarty et al., 2012 [57]	USA	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	796/501	≥18	Never smoker	-
Missmer et al., 2004 [13]	USA	Cohort (Nurese Health Study II)	Women with self-reported endometriosis	Women aged without self-reported endometriosis	1721/88344	25-52	Never, former, current smoker (1-14, 15-24, 25-34, ≥35 cigarettes/day)	Age, calendar time, race, parity, body mass index at 18, alcohol drinking
Moen et al., 1997 [58]	Norway	Case-control	Women with self-reported endometriosis	Women aged without self-reported endometriosis	79/3955	40-42	Current smoker	-

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Niskar et al., 2009 [59]	USA	Case-control	Nulliparous women seeking reproductive assistance with endometriosis (laparoscopically confirmed)	Nulliparous women seeking reproductive assistance without endometriosis	60/64	20-45	Ever smoker	-
Parazzini et al., 2008 [60]	Italy	Case-control	Women with deep endometriosis or pelvic and ovarian endometriosis (laparoscopically confirmed); separate groups of deep endometriosis and pelvic and ovarian endometriosis	Women without endometriosis admitted to hospital for acute non-gynecological, non-hormonal, non-neoplastic conditions, participating as controls in a case-control study on female genital neoplasms	181 + 162/329	20-55	Never, former, current	-
Pauwels et al., 2001 [61]	Belgium	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	42/27	24-42	Non smokers	-
Porpora et al., 2009 [62]	Italy	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis who underwent laparoscopy for benign gynecological conditions (unrelated to infertility)	80/78	18-45	Never, former, current smokers (1-9, 10-19, ≥20 cigarettes/day)	-
Sangi-Haghpeykar et al., 1995 [63]	USA	Case-control	Women undergoing laparoscopic tubal sterilization with endometriosis	Women undergoing laparoscopic tubal sterilization without endometriosis	126/504	NA	Never, former, current smoker (< 1 pack/day, ≥ 1 pack/day)	Age, number of live births

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors
Signorello et al., 1997 [14]	USA	Case-control	Women with infertility-associated endometriosis (laparoscopically confirmed)	fertile and infertile women both without endometriosis (laparoscopically confirmed); separate groups of fertile and infertile controls	50/89 + 47	23-44	Never, ever smoker	-
Treloar et al., 2010 [64]	Australia	Case-control	Women with endometriosis (surgically confirmed) with no first degree relative with endometriosis	Same-sex female twin pairs enrolled with the Australian Twin Registry, without endometriosis (self-reported)	268/244	18-55	Never, former, current smoker	-
Tsuchiya et al., 2007 [65]	Japan	Case-control	Women who had not given birth or lactate, with endometriosis (laparoscopically confirmed); separate groups of stage I/II and stage III/IV endometriosis	Women who had not given birth or lactate without endometriosis (laparoscopically confirmed)	79/59	20-45	Never, ever smoker	-

NA: not available; NHANES: National Health and Nutrition Examination Survey; OC: oral contraceptiv

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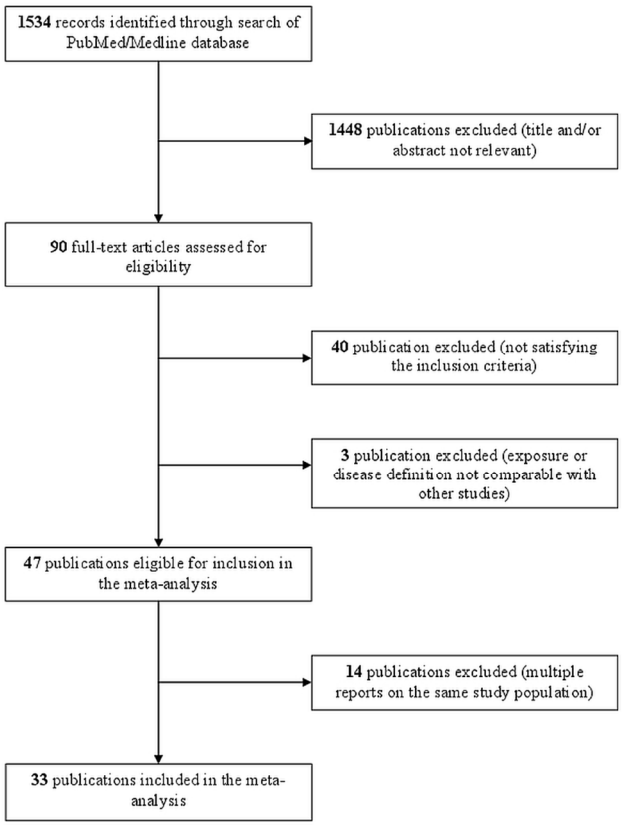

Figure 1 – Flow chart of the selection of studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

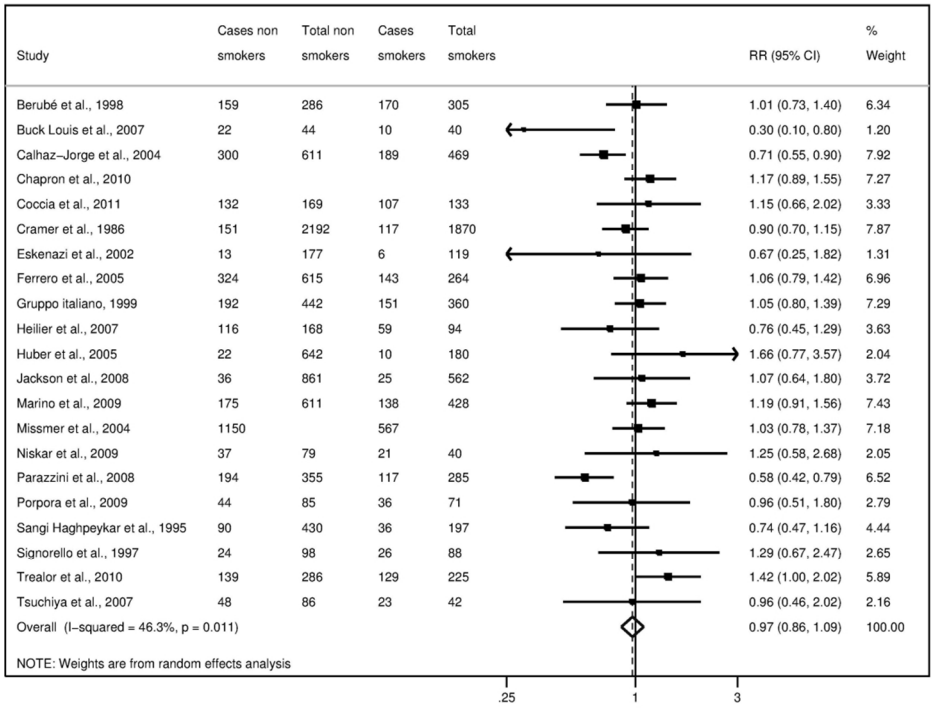

Figure 2 – Study-specific and summary relative risks (RR) of endometriosis for ever smokers versus non smokers.

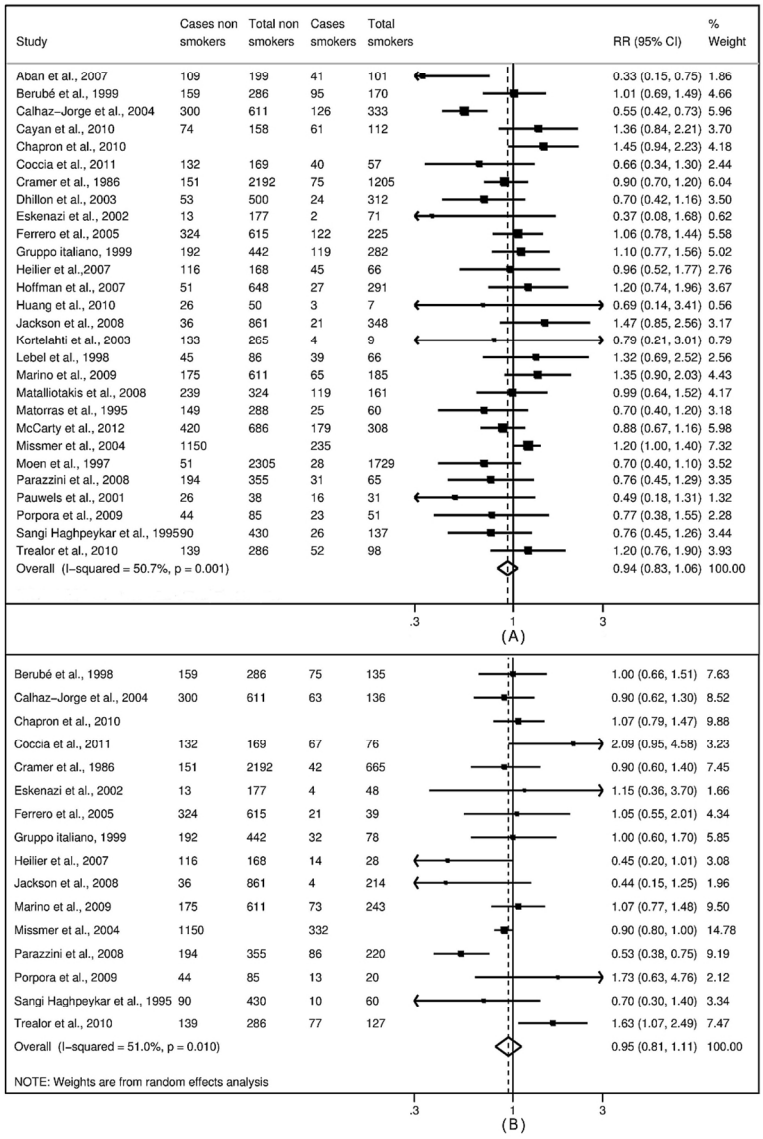

Figure 3 – Study-specific and summary relative risks (RR) of endometriosis for current (A) and former smokers (B) versus non smokers.
CI: confidence interval.

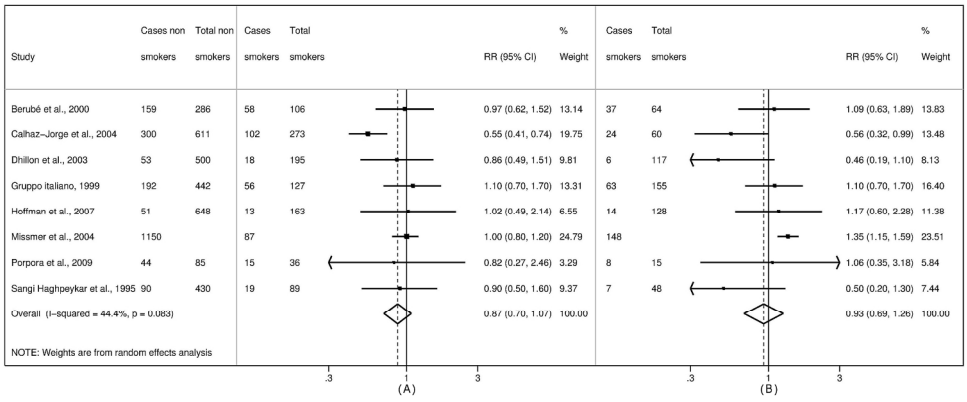

Figure 4 – Study-specific and summary relative risks (RR) of endometriosis for moderate (A) and heavy (B) current smokers versus non smokers.
CI: confidence interval.

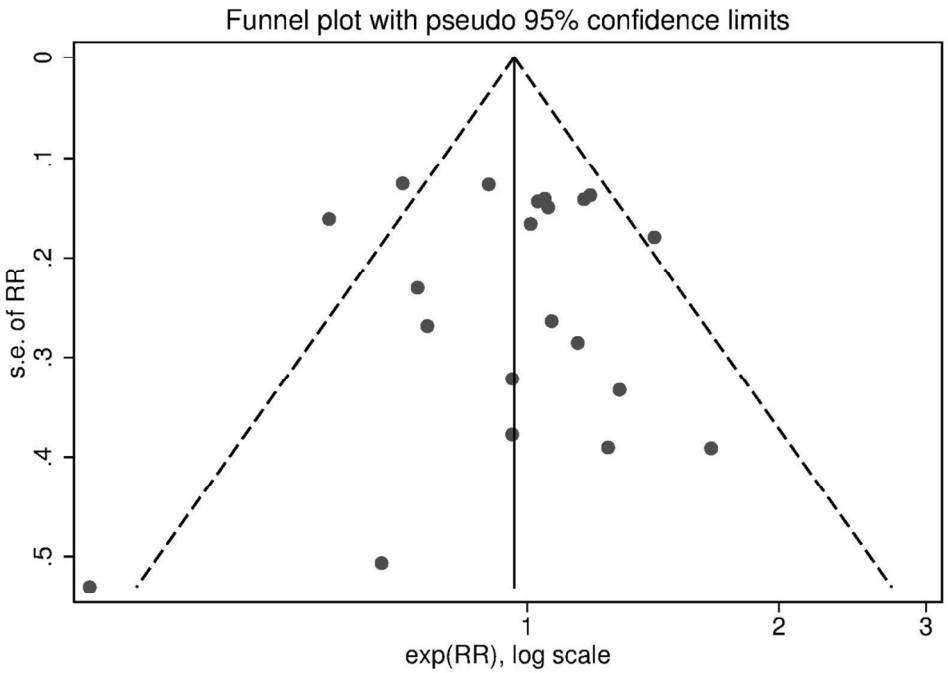

Figure 5 – Funnel-plot of studies on tobacco smoking and risk of endometriosis.
RR: relative risk for ever smokers versus non smokers; CI: confidence interval; s.e.: standard error

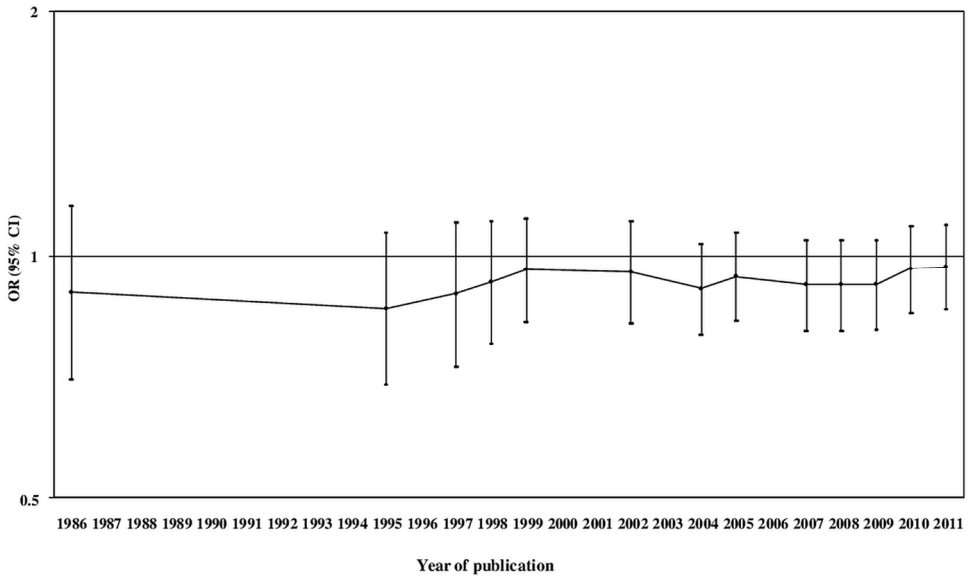

Figure 6 - Cumulative meta-analysis of studies on tobacco smoking and risk of endometriosis. RR: relative risk for coffee consumption versus no consumption; CI: confidence interval.

MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies*

Title	Identify the study as a meta-analysis (or systematic review)
Abstract	Use the journal's structured format
Introduction	**Present** • The clinical problem • The hypothesis • A statement of objectives that includes the study population, the condition of interest, the exposure or intervention, and the outcome(s) considered
Sources	**Describe** • Qualifications of searchers (eg, librarians and investigators) • Search strategy, including time period included in the synthesis and keywords • Effort to include all available studies, including contact with authors • Databases and registries searched • Search software used, name and version, including special features used (eg, explosion) • Use of hand searching (eg, reference lists of obtained articles) • List of citations located and those excluded, including justification • Method of addressing articles published in languages other than English • Method of handling abstracts and unpublished studies • Description of any contact with authors
Study Selection	**Describe** • Types of study designs considered • Relevance or appropriateness of studies gathered for assessing the hypothesis to be tested • Rationale for the selection and coding of data (eg, sound clinical principles or convenience) • Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability) • Assessment of confounding (eg, comparability of cases and controls in studies where appropriate) • Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results • Assessment of heterogeneity • Statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated
Results	**Present** • A graph summarizing individual study estimates and the overall estimate • A table giving descriptive information for each included study • Results of sensitivity testing (eg, subgroup analysis) • Indication of statistical uncertainty of findings
Discussion	**Discuss** • Strengths and weaknesses • Potential biases in the review process (eg, publication bias) • Justification for exclusion (eg, exclusion of non–English-language citations) • Assessment of quality of included studies • Consideration of alternative explanations for observed results • Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review) • Guidelines for future research • Disclosure of funding source

*Modified from Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008–12.

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Tobacco smoking and risk of endometriosis: a systematic review and meta-analysis

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Tobacco smoking and risk of endometriosis: a systematic review and meta-analysis

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Keywords: endometriosis; tobacco smoking; meta-analysis.

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Abbreviations: CI: confidence interval, HR: hazard ratio, MOOSE: meta-analysis of observational studies in epidemiology, OR: odds ratio, RR: relative risk.

ABSTRACT

Objective: Since conflicting results have been published on the role of tobacco smoking on the risk of endometriosis, we provide an up to date summary quantification of this potential association.

Design: We performed a PubMed/MEDLINE search of the relevant publications up to September 2014, considering studies on humans published in English. We searched the reference list of the identified papers to identify other relevant publications. Both case-control or cohort studies have been included reporting risk estimates on the association between tobacco smoking and endometriosis. Thirty-eight out of the 1,758 screened papers met the inclusion criteria. The selected studies included a total of 13,129 women diagnosed with endometriosis.

Setting: Academic hospitals

Main outcome measures: Risk of endometriosis in tobacco smokers.

Results: We obtained the summary estimates of the relative risk (RR) using the random-effect model, and assessed the heterogeneity among studies using the χ^2 test and quantified it using the I^2 statistic. As compared to never smokers, the summary RR were 0.96 (95% confidence interval, CI: 0.86-1.08) for ever smokers, 0.93 (95% CI: 0.77-1.12) for former smokers, 0.94 (95% CI:0.81-1.10) for current smokers, 0.87 (95% CI: 0.70-1.07) for moderate smokers, and 0.93 (95% CI: 0.69-1.26) for heavy smokers.

Conclusions: The present meta-analysis provided no evidence for an association between tobacco smoking and the risk of endometriosis. The results were consistent considering ever, former, current, moderate, and heavy smokers, and across type of endometriosis and study design.

Strengths and limitations of the study

- Meta-analysis including 38 papers without any relevant asymmetry in the funnel plot.
- The Egger's test was not statistically significant.
- In some studies, choice of the cases as asymptomatic without distinguishing factors related to endometriosis to those associated to pelvic pain or infertility.
- In some studies, choice of controls in whom disease was not laparoscopically ruled out.
- Tobacco smoking based on patients' self-reported information.

INTRODUCTION

Endometriosis is an estrogen-dependent, chronic inflammatory gynecological condition characterized by the proliferation of functional endometrial tissue that develops outside the uterine cavity, which may cause pain and infertility [1]. However, despite its relatively high prevalence, which spans from 20% in asymptomatic women [2], to 30% in women with infertility [3], and 45% in women with pain symptoms [4], risk factors for this condition remain largely unknown.

Among the risk factors investigated, some studies have examined the role of tobacco smoking. In a Portuguese study investigating clinical and lifestyle factors in infertile women, current smokers had a decreased risk of endometriosis as compared to non-smokers or former smokers [5]. In a case-control study from Turkey evaluating the interaction between tobacco smoking and glutathione-S-transferase gene polymorphism as a risk factor for endometriosis, an inverse association between smoking and endometriosis was observed [6]. In a case-control study carried out in the USA, infertile women with endometriosis and fertile controls were compared and a decreased risk of endometriosis was found, though limited to women who begun smoking at an early age and were heavy smokers [7]. Other studies did not find significant association [3, 8-14].

The biological plausibility potentially linking smoking and endometriosis resides in its endocrine and inflammatory mechanisms. Smoke compounds disrupt steroidogenesis, leading to impairment of E2 synthesis [15, 16] and progesterone synthesis deficiency [17-19]. Moreover, smoking has a strong effect on inflammatory mediators in both the pulmonary and extra-pulmonary environments and can further trigger inflammation associated with the disease resulting in pro-inflammatory gene overexpression [20].

A clear definition of the relation between smoking and endometriosis risk has an interest in order to better understand the role of estrogens, in consideration of the potential anti estrogenic effect of smoking. Otherwise in clinical term, a direct association as reported in some studies [6, 7] may suggest preventive measures.

Thus, in order to investigate the possible relation between tobacco smoking and endometriosis, and to provide an overall quantitative estimate of any such relation, we combined in a meta-analysis all published data on the issue.

MATERIALS AND METHODS

Search strategy

We performed a PubMed/MEDLINE search of papers published between 1966 and September 2014, using the terms "tobacco" or "smoking" or "cigarette" in combination with "risk factor", or "epidemiology", and "endometriosis", following the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines [21]; details on the search terms are provided in Appendix. We selected only studies on humans, published as full-length papers in English. No effort was made to identify papers published in other languages or unpublished studies. Moreover, we reviewed the reference lists of the retrieved papers, to identify any other relevant publication. Studies were included in the meta-analysis if: a) they were based on case-control or cohort studies, reporting original data; b) they reported information on the association between tobacco smoking and endometriosis, including estimates of the relative risk (RR) (approximated by the odds ratio, OR, in case-control studies) , with the corresponding 95% confidence intervals (CI), or frequency distribution to calculate them; c) diagnosis of endometriosis was histologically confirmed and/or clinically based. When we found more than one publication based on the same study population and data, we included only the one with most detailed information, or published most recently.

We used the Newcastle-Ottawa Scale [22] to assess the quality of individual studies and performed a sensitivity analysis according to the quality of each study.

Data extraction for the meta-analysis

Two authors (FB and SC) reviewed the manuscripts and independently selected the eligible manuscripts; disagreements were resolved by discussion. From each publication we extracted the

following information: country of origin; study design; number and characteristics of subjects (cases, controls or cohort size); age, if available; categories of tobacco smoking, if available; measures of association (RR, or OR) of endometriosis and corresponding 95% CI for every category of tobacco smoking, or frequency distribution to calculate them; confounding variables allowed for in the statistical analysis, if any. When more than one regression model was provided, estimates adjusted for the largest number of confounding variables were considered.

Statistical analysis

For some studies, we pooled estimates of different categories of cases or controls using the method by Hamling et al. [23], which allows to combine the estimates originally shown in the paper, changing the reference category, and taking into account their correlation. We obtained the summary estimates of the RR using the random-effect model (i.e., as weighed averages on the sum of the inverse of the variance of the log RR and the moment estimator of the variance between studies) [24]. We assessed the heterogeneity among studies using the χ^2 test [25] and quantified it using the I^2 statistic, which represents the percentage of the total variation across studies that is attributable to heterogeneity rather than chance [26]. Results were defined as heterogeneous for P values less than 0.10.

We computed summary estimates for ever tobacco smokers, former smokers, current smokers, moderate current smokers, and heavy current smokers, as compared to never smokers. Different cut-points for moderate and heavy smoking were chosen, depending on those shown in the papers. We also carried out a cumulative meta-analysis to determine whether the association between tobacco smoking and endometriosis changed over time. In the cumulative meta-analysis studies are added one at a time, ordered by year of publication, and the results are pooled as each new study is added. In the graph the vertical line corresponding to each year represents the RR and corresponding CI of the results of the meta-analysis of the studies published up to that year, rather than the results of a single study [27]. Furthermore, we performed subgroup analyses according to

type of controls (fertile, infertile, both/not specified). Publication bias was evaluated using funnel plot [28] and was quantified by the Egger's test [29].

RESULTS

Figure 1 shows the flow-chart of the selection of publications. The literature search yielded 1,758, 1,620 of which were excluded after evaluation of abstract and full text, because did not report any information on the relationship between tobacco smoking and risk of endometriosis, and 80 because did not satisfy the inclusion criteria. Moreover, 4 studies were not comparable with the other ones, since reported estimates for lifetime smoking [30], included former or light smokers in the reference category [11], included women with stage I endometriosis in the comparison group [31], or reported serum cotinine as measure of exposure to tobacco smoking (including passive smoking as well) [32], and thus we excluded those studies from the meta-analysis.

Furthermore, we excluded 16 studies based on the same data of other included publications [33-48]. Thus, in the present meta-analysis we combined data from 38 studies, including a total of 13,129 women with endometriosis (suppl. File Table 1) [3, 5-10, 12-14, 49-76].

Table 1 shows the main characteristics of the studies included in the present meta-analysis. Most publications were based on case-control studies, while 9 were cohort studies, in which, however, the role of smoking was evaluated at the same time of the disease diagnosis [13, 50, 52, 54, 58, 70, 74], except in two cases, in which smoking status was assessed at baseline [5, 49]. Of these, 16 studies were from Europe [3, 5, 9, 10, 49, 52, 54-57, 60, 62, 66, 68, 69, 71], 13 from the USA [7, 12-14, 50, 53, 58, 61, 64, 65, 67, 70, 72], 2 from Canada [8, 63], 5 from Asia [6, 51, 59, 74, 75], and 2 from Australia [73, 76].

Twenty-four studies reported information on ever smokers [5, 7-10, 13, 14, 49, 50, 52, 54, 56, 57, 60, 61, 64, 67, 68, 71-76], 16 on former smokers [5, 7-10, 13, 52, 54, 56, 57, 61, 64, 68, 71-73], and 30 on current smokers [3, 5-10, 12, 13, 51-59, 61-66, 68-73]. Among these, 8 reported more categories of current smokers, thus we could calculate separate estimates for moderate and heavy current smokers. We used different cut-points for various study populations, depending on those presented in the papers: thus the cut-point between moderate

and heavy smokers were defined as 20 cigarettes per day in 5 studies [5, 8, 53, 71, 72], 15 cigarettes per day in 2 studies [13, 58] and 10 cigarettes per day in 1 study [10].

For some studies reporting separate estimates for different types of patients and/or controls, we computed a pooled estimate. In particular, Coccia et al. [52] reported separate estimates for monolateral and bilateral endometriosis, Heilier et al. [57] for endometriosis and deep endometriotic nodules, Parazzini et al. [68] for deep endometriosis and pelvic and ovarian endometriosis, Signorello et al. [14] for fertile and infertile controls, Tsuchiya et al. [75] for stage I/II and stage III/IV endometriosis. Moreover, Calahz-Jorge et al. [5] reported separate estimates for grade I/II and grade III/IV endometriosis, as well as for any type of endometriosis, and the Gruppo Italiano per lo Studio dell'endometriosi [10], including two separate groups of cases and controls undergoing laparoscopy for pelvic pain or infertility, showed both separate and pooled estimate; in both cases we included in the meta-analysis the combined estimates; further, Pollack et al. included an operative cohort comprising women scheduled for laparoscopy/laparotomy and an aged-matched population cohort of women who underwent pelvic magnetic resonance for the detection of endometriosis, and we summed up the two groups [70].

Considering ever smokers or separately former smokers, current smokers, moderate smokers and heavy smokers, no statistically significant association emerged (Figures 2-4).

Figure 5 shows the funnel plot for ever smokers versus non smokers. There was no evidence of publication bias (p=0.054).

When we restricted the analyses to 9 studies reporting risk estimates adjusted for confounding variables, risk estimates were 1.01 (95% CI: -0.86-1.19) for ever smokers, 0.94 (95% CI: 0.85-1.03) for former smokers, 0.87 (95% CI: 0.64-1.17) for current smokers, 0.85 (95% CI:0.60-1.20) for moderate current smokers, and 0.90 (95% CI: 0.57-1.43) for heavy current smokers versus never smokers.

In subgroup analyses according to type of controls, estimates for ever versus non smokers were 1.06 (95% CI:0.89-1.27) for 7 studies including fertile women, 0.92 (95% CI: 0.75-1.12) for 7 studies including infertile women, and 0.95 (95% CI:0.81-1.12) for 14 studies including both or not specified type of controls. Moreover, when we restricted the analyses to studies with cases and controls laparoscopically or surgically confirmed, the risk estimates were 0.97 (95% CI:0.87-1.07) for ever smokers, 0.94 (95% CI: 0.85-1.03) for former smokers, 0.90 (95 % CI:0.77-1.04) for current smokers, 0.86 (95% CI: 0.66-1.12) for moderate smokers, and 0.97 (95% CI: 0.70-1.35) for heavy smokers.

Quality score, ranged between 2 and 7 (median 4.5). When we restricted the meta-analysis to 19 high quality studies (with quality score≥5) the pooled estimates did not materially changed (data not shown).Figure 6 shows the cumulative meta-analysis of endometriosis risk for ever smokers versus non smokers over time, from 1986 to2014: small variations over time in the RR estimates emerged.

DISCUSSION

The present meta-analysis does not support an association between smoking and endometriosis risk. No association emerged considering subgroups of ever, former, current, moderate and heavy smokers, nor in sensitivity and subgroup analyses

However, this work may be affected by limitations and biases intrinsic in the original observational studies included in the meta-analysis, as well as to the limits that we choose to apply to the bibliographic search, including the restriction to searching PubMed only and the exclusion of languages other than English. As regards the characteristics of the observational studies, a major concern is ascertainment of the presence or absence of endometriosis. Some studies compared symptomatic cases with asymptomatic controls, and thus could not distinguish factors related to endometriosis to those associated to pelvic pain or infertility. Moreover, generally asymptomatic controls did not undergo laparoscopy nor other surgical procedures, and therefore the presence of

asymptomatic endometriosis in these women cannot be ruled out. Another concern is the fact that in some studies diagnosis of endometriosis was self reported. Thus, a misclassification of cases and controls could not be definitively excluded. However, when we restricted the analyses to women in whom laparoscopy or a surgical procedure had confirmed the presence or absence of endometriotic lesions, still we did not find any significant association between smoking and endometriosis. Further, tobacco smoking is based on patients' self-reported information, thus some misclassification may have occurred. However, information on tobacco smoking in observational studies has been shown to be satisfactorily reproducible and valid [77-79]. For most studies included in the present meta-analysis only raw estimates were available, since tobacco smoking was not the main topic of the paper and it was only reported as confounding variable. However, estimates from these studies were similar to those from studies specifically investigating the role of smoking, thus, allowing to rule out major publication bias on this issue. Moreover, we did not find any relevant asymmetry in the funnel plot, and the Egger's test was not statistically significant. Thus, publication bias is unlikely to have appreciably modified the relation between tobacco smoking and endometriosis. Although previous studies have reported an association between endometriosis and menstrual and reproductive factors, such as early menarche [7, 12], longer duration of bleeding [7], intra-uterine device use [80], or a lifelong regular menstrual pattern of shorter cycles and heavy flows [7, 12, 72, 81], nulliparity or low parity [14, 30, 38, 82], only some studies included in the present meta-analysis have accounted for the role of these factors in the estimate of the relation between tobacco smoking and endometriosis. However, analyses based on adjusted estimates only were comparable to those based on raw estimates.

Since endometriosis is an estrogen-dependent condition, the inverse association between smoking and endometriosis found in some studies has generally been attributed to the antiestrogenic effect of tobacco [83]. Some authors have suggested that estradiol might modulate the mediators of immune system molecules or those involved in tissue cell adhesion and invasion [84, 85]. Moreover, a favorable

effect of smoking has been observed in other benign and malignant estrogen-related diseases, such as endometrial cancer [86], and fibroids [87]. The antiestrogenic effect of smoking on these conditions could support a protective effect of smoking on endometriosis. Indeed, earlier studies tended to support some inverse association, which however declined over time, and accumulating evidence suggests the presence of some false positive findings in earlier studies [88]. Furthermore, tobacco smoking has been associated with female infertility [89], and thus the interpretation of the relation between smoking and endometriosis may be influenced by the role of infertility.

Despite the high prevalence of this condition, the epidemiology of endometriosis still needs to be elucidated, for several reasons. Endometriosis is a complex condition in which a genetic contribution and environmental factors seem to be involved [90]. Further, it is a disease characterized by a still poorly defined phenotype. The disease stage depends on the type (cysts, implants, nodules), location (ovary, peritoneum, bladder, ureter, etc.), appearance and depth of invasion of the lesions, that can vary greatly among patients. The clinical presentation can be so variable and the lesions of such diverse morphology that none of the pathogenetic models proposed (retrograde menstruation, coelomic metaplasia, embryological origin) can fully explain the various aspects of endometriosis, and none has been recognized as an ultimately valid explanatory model for all the different forms and manifestations of the disease [90]. Moreover, an invasive procedure is needed to diagnose it [90, 91]. Furthermore, published studies differ in the case and control selection and population definition, depending on the choices to consider fertile or infertile cases, and healthy controls or patients with conditions other than endometriosis. Despite these possible sources of variation, the consistency of results observed weighs against any relevant role of tobacco on endometriosis.

In conclusion, the present meta-analysis failed to identify an association between tobacco smoking and endometriosis. However, given the possible limitations of the present study, further studies are

needed to evaluate in deep the relationship and the potential effect of smoking on different type of endometriosis.

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Contributors

FP conceived the idea and planned the research. FB and SC performed the statistical analysis. FB, FC, ER, VC retrieved data. FP, FB, PV and CLV wrote the entire draft of the article and all subsequent drafts after critical review by all co-authors. All co-authors gave significant input in the preparation of the article and the analysis. FP is the guarantor for the article.

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FIGURE LEGENDS

Figure 1 – Flow chart of the selection of studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

Figure 2 – Study-specific and summary relative risks (RR) of endometriosis for ever smokers versus non smokers.

CI: confidence interval.

Figure 3 – Study-specific and summary relative risks (RR) of endometriosis for current (A) and former smokers (B) versus non smokers.

CI: confidence interval.

Figure 4 – Study-specific and summary relative risks (RR) of endometriosis for moderate (A) and heavy (B) current smokers versus non smokers.

CI: confidence interval.

Figure 5 – Funnel-plot of studies on tobacco smoking and risk of endometriosis.

RR: relative risk for ever smokers versus non smokers; CI: confidence interval; s.e.: standard error.

Figure 6 - Cumulative meta-analysis of studies on tobacco smoking and risk of endometriosis.

RR: relative risk for ever smokers versus non smokers; CI: confidence interval.

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Tobacco smoking and risk of endometriosis: a systematic review and meta-analysis

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Abbreviations: CI: confidence interval, HR: hazard ratio, MOOSE: meta-analysis of observational studies in epidemiology, OR: odds ratio, RR: relative risk.

ABSTRACT

Objective: Since conflicting results have been published on the role of tobacco smoking on the risk of endometriosis, we provide an up to date summary quantification of this potential association.

Design: We performed a PubMed/MEDLINE search of the relevant publications up to ~~May 2012~~ September 2014, considering studies on humans published in English. We searched the reference list of the identified papers to identify other relevant publications. Both case-control or cohort studies have been included reporting risk estimates on the association between tobacco smoking and endometriosis. Thirty-~~three~~eight out of the ~~1,534~~1,758 screened papers met the inclusion criteria. The selected studies included a total of ~~8,225~~13,129 women diagnosed with endometriosis.

Setting: Academic hospitals

Main outcome measures: Risk of endometriosis in tobacco smokers.

Results: We obtained the summary estimates of the relative risk (RR) using the random-effect model, and assessed the heterogeneity among studies using the χ^2 test and quantified it using the I^2 statistic. As compared to never smokers, the summary RR were ~~0.97~~0.96 (95% confidence interval, CI: ~~0.86-1.09~~0.86-1.08) for ever smokers, ~~0.95~~0.93 (95% CI: ~~0.81-1.11~~0.77-1.12) for former smokers, 0.94 (95% CI: ~~0.83-1.06~~0.81-1.10) for current smokers, 0.87 (95% CI: 0.70-1.07) for moderate smokers, and 0.93 (95% CI: 0.69-1.26) for heavy smokers.

Conclusions: The present meta-analysis provided no evidence for an association between tobacco smoking and the risk of endometriosis. The results were consistent considering ever, former, current, moderate, and heavy smokers, and across type of endometriosis and study design.

Strengths and limitations of the study

- Meta-analysis including ~~33~~ 38 papers without any relevant asymmetry in the funnel plot.
- The Egger's test was not statistically significant.
- In some studies, choice of the cases as asymptomatic without distinguishing factors related to endometriosis to those associated to pelvic pain or infertility.
- In some studies, choice of controls in whom disease was not laparoscopically ruled out.
- Tobacco smoking based on patients' self-reported information.

INTRODUCTION

Endometriosis is an estrogen-dependent, chronic inflammatory gynecological condition characterized by the proliferation of functional endometrial tissue that develops outside the uterine cavity, which may cause pain and infertility [1]. However, despite its relatively high prevalence, which spans from 20% in asymptomatic women [2], to 30% in women with infertility [3], and 45% in women with pain symptoms [4], risk factors for this condition remain largely unknown.

Among the risk factors investigated, some studies have examined the role of tobacco smoking. In a Portuguese study investigating clinical and lifestyle factors in infertile women, current smokers had a decreased risk of endometriosis as compared to non-smokers or former smokers [5]. In a case-control study from Turkey evaluating the interaction between tobacco smoking and glutathione-S-transferase gene polymorphism as a risk factor for endometriosis, an inverse association between smoking and endometriosis was observed [6]. In a case-control study carried out in the USA, infertile women with endometriosis and fertile controls were compared and a decreased risk of endometriosis was found, though limited to women who begun smoking at an early age and were heavy smokers [7]. Other studies did not find significant association [3, 8-14].

The biological plausibility potentially linking smoking and endometriosis resides in its endocrine and inflammatory mechanisms. Smoke compounds disrupt steroidogenesis, leading to impairment of E2 synthesis [15, 16] and progesterone synthesis deficiency [17-19]. Moreover, smoking has a strong effect on inflammatory mediators in both the pulmonary and extra-pulmonary environments and can further trigger inflammation associated with the disease resulting in pro-inflammatory gene overexpression [20].

A clear definition of the relation between smoking and endometriosis risk has an interest in order to better understand the role of estrogens, in consideration of the potential anti estrogenic effect of smoking. Otherwise in clinical term, a direct association as reported in some studies [6, 7] may suggest preventive measures.

Thus, in order to investigate the possible relation between tobacco smoking and endometriosis, and to provide an overall quantitative estimate of any such relation, we combined in a meta-analysis all published data on the issue.

MATERIALS AND METHODS

Search strategy

We performed a PubMed/MEDLINE search of papers published between 1966 and ~~May 2012~~ September 2014, using the terms "tobacco" or "smoking" or "cigarette" in combination with "risk factor", or "epidemiology", and "endometriosis", following the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines [21]; details on the search terms are provided in Appendix. We selected only studies on humans, published as full-length papers in English. No effort was made to identify papers published in other languages or unpublished studies. Moreover, we reviewed the reference lists of the retrieved papers, to identify any other relevant publication. Studies were included in the meta-analysis if: a) they were based on case-control or cohort studies, reporting original data; b) they reported information on the association between tobacco smoking and endometriosis, including estimates of the relative risk (RR) (approximated by the odds ratio, OR, in case-control studies) ~~or the odds ratio (OR) or the hazard ratio (HR)~~, with the corresponding 95% confidence intervals (CI), or frequency distribution to calculate them; c) diagnosis of endometriosis was histologically confirmed and/or clinically based. When we found more than one publication based on the same study population and data, we included only the one with most detailed information, or published most recently.

We used the Newcastle-Ottawa Scale [22] to assess the quality of individual studies and performed a sensitivity analysis according to the quality of each study.

Data extraction for the meta-analysis

Two authors (FB and SC) reviewed the manuscripts and independently selected the eligible manuscripts; disagreements were resolved by discussion. From each publication we extracted the following information: country of origin; study design; number and characteristics of subjects (cases, controls or cohort size); age, if available; categories of tobacco smoking, if available; measures of association (RR, or OR ~~or HR~~) of endometriosis and corresponding 95% CI for every category of tobacco smoking, or frequency distribution to calculate them; confounding variables allowed for in the statistical analysis, if any. When more than one regression model was provided, estimates adjusted for the largest number of confounding variables were considered.

Statistical analysis

For some studies, we pooled estimates of different categories of cases or controls using the method by Hamling et al. [23], which allows to combine the estimates originally shown in the paper, changing the reference category, and ~~thus~~ taking into account their correlation. We obtained the summary estimates of the RR using the random-effect model (i.e., as weighed averages on the sum of the inverse of the variance of the log RR and the moment estimator of the variance between studies) [24]. We assessed the heterogeneity among studies using the χ^2 test [25] and quantified it using the I^2 statistic, which represents the percentage of the total variation across studies that is attributable to heterogeneity rather than chance [26]. Results were defined as heterogeneous for P values less than 0.10.

We computed summary estimates for ever tobacco smokers, former smokers, current smokers, moderate current smokers, and heavy current smokers, as compared to never smokers. Different cut-points for moderate and heavy smoking were chosen, depending on those shown in the papers.

We also carried out a cumulative meta-analysis to determine whether the association between tobacco smoking and endometriosis changed over time. In the cumulative meta-analysis studies are added one at a time, ordered by year of publication, and the results are pooled as each new study is added. In the graph the vertical line corresponding to each year represents the RR and

corresponding CI of the results of the meta-analysis of the studies published up to that year, rather than the results of a single study [27]. ~~and performed~~ Furthermore, we performed subgroup analyses according to type of controls (fertile, infertile, both/not specified). Publication bias was evaluated using funnel plot [28] and was quantified by the Egger's test [29].

RESULTS

Figure 1 shows the flow-chart of the selection of publications. ~~From t~~The literature search ~~we identified~~ yielded 1,758~~1534 studies~~, ~~1448~~ 1,620 of which were excluded ~~because not relevant~~after evaluation of abstract and full text, because did not report any information on the relationship between tobacco smoking and risk of endometriosis, and ~~40~~80 because did not satisfy the inclusion criteria. Moreover, ~~3~~4 studies were not comparable with the other ones, since reported estimates for lifetime smoking [30], included former or light smokers in the reference category [11], ~~or~~ included women with stage I endometriosis in the comparison group [31], or reported serum cotinine as measure of exposure to tobacco smoking (including passive smoking as well) [32], and thus we excluded those studies from the meta-analysis.

Furthermore, we excluded ~~14~~16 studies based on the same data of other included publications [33-48]. Thus, in the present meta-analysis we combined data from ~~33~~38 studies, including a total of ~~8225~~13,129 women with endometriosis (suppl. File Table 1) [3, 5-10, 12-14, 49-76].

Table 1 shows the main characteristics of the studies included in the present meta-analysis. Most publications were based on case-control studies, while ~~six~~9 were cohort studies, in which, however, the role of smoking was ~~not~~ evaluated ~~prospectively~~ at the same time of the disease diagnosis [13, 50, 52, 54, 58, 70, 74], except in ~~one~~ two cases, in which smoking status was assessed at baseline [5, 49]. Of these, ~~14~~16 studies were from Europe [3, 5, 9, 10, 49, 52, 54-57, 60, 62, 66, 68, 69, 71], ~~12~~13 from the USA [7, 12-14, 50, 53, 58, 61, 64, 65, 67, 70, 72], 2 from Canada [8, 63], ~~4~~5 from Asia [6, 51, 59, 74, 75], and ~~1~~2 from Australia [73, 76].

Twenty-~~one~~ four studies reported information on ever smokers [5, 7-10, 13, 14, 49, 50, 52, 54, 56, 57, 60, 61, 64, 67, 68, 71-76], 16 on former smokers [5, 7-10, 13, 52, 54, 56, 57, 61, 64, 68, 71-73], and ~~28~~30 on current smokers [3, 5-10, 12, 13, 51-59, 61-66, 68-73]. Among these, 8 reported more categories of current smokers, thus we could calculate separate estimates for moderate and heavy current smokers. We used different cut-points for various study populations, depending on those presented in the papers: thus the cut-point between moderate and heavy smokers were defined as 20 cigarettes per day in 5 studies [5, 8, 53, 71, 72], 15 cigarettes per day in 2 studies [13, 58] and 10 cigarettes per day in 1 study [10].

For some studies reporting separate estimates for different types of patients and/or controls, we computed a pooled estimate. In particular, Coccia et al. [52] reported separate estimates for monolateral and bilateral endometriosis, Heilier et al. [57] for endometriosis and deep endometriotic nodules, Parazzini et al. [68] for deep endometriosis and pelvic and ovarian endometriosis, Signorello et al. [14] for fertile and infertile controls, Tsuchiya et al. [75] for stage I/II and stage III/IV endometriosis. Moreover, Calahz-Jorge et al. [5] reported separate estimates for grade I/II and grade III/IV endometriosis, as well as for any type of endometriosis, and the Gruppo Italiano per lo Studio dell'endometriosi [10], including two separate groups of cases and controls undergoing laparoscopy for pelvic pain or infertility, showed both separate and pooled estimate; in both cases we included in the meta-analysis the combined estimates; further, Pollack et al. included an operative cohort comprising women scheduled for laparoscopy/laparotomy and an aged-matched population cohort of women who underwent pelvic magnetic resonance for the detection of endometriosis, and we summed up the two groups [70].

~~Figure 2 shows the study-specific and summary RRs of endometriosis for ever smokers versus non smokers. The summary RR from studies was 0.97 (95% CI: 0.86-1.09)(x^2 heterogeneity between studies =37.23, p=0.011). Figure 3 gives the study-specific and summary RR of current (A) and former (B) smokers versus never smokers. The summary RR of current versus never smokers was 0.94 (95% CI: 0.83-1.06) from 28 studies (x^2 heterogeneity =54.76, p=0.001). The summary RR of~~

~~former versus never smokers was 0.95 (95% CI: 0.81-1.11) from 16 studies, with hetergogeneity (x^2=30.63, p=0.010). Figure 4 shows the RR of moderate (A) and heavy (B) current smokers versus non smokers, respectively. The summary RR from 8 studies were 0.87 (95% CI: 0.70-1.07)(x^2 heterogeneity =12.58, p=0.083), and 0.93 (95% CI: 0.69-1.26)(x^2 heterogeneity =17.21, p=0.016), for moderate and heavy smokers, respectively.~~

Considering ever smokers or separately former smokers, current smokers, moderate smokers and heavy smokers, no statistically significant association emerged (Figures 2-4).

Figure 5 shows the funnel plot for ever smokers versus non smokers. There was no evidence of publication bias (p=~~0.924~~0.054).

When we restricted the analyses to ~~8~~ 9 studies reporting risk estimates adjusted for confounding variables, risk estimates were ~~0.90~~1.01 (95% CI: ~~0.77-1.06~~0.86-1.19) for ever smokers, ~~0.87~~ 0.94 (95% CI: ~~0.75-1.01~~0.85-1.03) for former smokers, ~~0.86~~0.87 (95% CI: ~~0.71-1.06~~0.64-1.17) for current smokers, ~~0.87~~0.85 (95% CI: ~~0.65-1.15~~0.60-1.20) for moderate current smokers, and ~~0.95~~0.90 (95% CI: ~~0.66-1.37~~0.57-1.43) for heavy current smokers versus never smokers.

In subgroup analyses according to type of controls, estimates for ever versus non smokers were ~~0.97~~1.06 (95% CI: ~~0.81-1.17~~0.89-1.27) for 7 studies including fertile women, 0.92 (95% CI: 0.75-1.12) for ~~6~~7 studies including infertile women, and ~~0.99~~0.95 (95% CI: ~~0.83-1.19~~0.81-1.12) for ~~12~~14 studies including both or not specified type of controls. Moreover, when we restricted the analyses to studies with cases and controls laparoscopically or surgically confirmed, the risk estimates were ~~0.98~~0.97 (95% CI: ~~0.87-1.09~~0.87-1.07) for ever smokers, 0.94 (95% CI: 0.85-1.03) for former smokers, ~~0.91~~0.90 (95 % CI: ~~0.77-1.07~~0.77-1.04) for current smokers, 0.86 (95% CI: 0.66-1.12) for moderate smokers, and 0.97 (95% CI: 0.70-1.35) for heavy smokers.

Quality score, ranged between 2 and 7 (median 4.5). When we restricted the meta-analysis to 19 high quality studies (with quality score≥5) the pooled estimates did not materially changed (data not shown).

Figure 6 shows the cumulative meta-analysis of endometriosis risk for ever smokers versus non smokers over time, from 1986 to ~~2011~~2014~~:. The estimate was 0.90 (95% CI: 0.70-1.15) in 1986 and 0.97 (95% CI: 0.86-1.09), with a few~~ small variations over time~~, all the estimates being not significantly below unity~~ in the RR estimates emerged.

DISCUSSION

The present meta-analysis does not support an association between smoking and endometriosis risk. No association emerged considering subgroups of ever, former, current, moderate and heavy smokers, nor in sensitivity and subgroup analyses~~.~~

However, ~~T~~this work may be affected by limitations and biases intrinsic in the original observational studies included in the meta-analysis, as well as to the limits that we choose to apply to the bibliographic search, including the restriction to searching PubMed only and the exclusion of languages other than English. ~~A~~ As regards the characteristics of the observational studies, a major concern is ~~the choice of the comparison group~~ascertainment of the presence or absence of endometriosis. Some studies compared symptomatic cases with asymptomatic controls, and thus could not distinguish factors related to endometriosis to those associated to pelvic pain or infertility. Moreover, generally asymptomatic controls did not undergo laparoscopy nor other surgical procedures, and therefore the presence of asymptomatic endometriosis in these women cannot be ruled out. Another concern is the fact that in some studies diagnosis of endometriosis was self reported. Thus, a misclassification of cases and controls could not be definitively excluded.

However, when we restricted the analyses to women in whom laparoscopy or a surgical procedure had confirmed the presence or absence of endometriotic lesions, still we did not find any significant association between smoking and endometriosis~~ of concern is the fact that in some studies diagnosis of endometriosis was self reported~~. Further, tobacco smoking is based on patients' self-reported information, thus some misclassification may have occurred. However, information on tobacco

smoking in observational studies has been shown to be satisfactorily reproducible and valid [77-79]. For most studies included in the present meta-analysis only raw estimates were available, since tobacco smoking was not the main topic of the paper and it was only reported as confounding variable. However, estimates from these studies were similar to those from studies specifically investigating the role of smoking, thus, allowing to rule out major publication bias on this issue. Moreover, we did not find any relevant asymmetry in the funnel plot, and the Egger's test was not statistically significant. Thus, publication bias is unlikely to have appreciably modified the relation between tobacco smoking and endometriosis. Although previous studies have reported an association between endometriosis and menstrual and reproductive factors, such as early menarche [7, 12], longer duration of bleeding [7], intra-uterine device use [80], or a lifelong regular menstrual pattern of shorter cycles and heavy flows [7, 12, 72, 81], nulliparity or low parity [14, 30, 38, 82], only some studies included in the present meta-analysis have accounted for the role of these factors in the estimate of the relation between tobacco smoking and endometriosis. However, analyses based on adjusted estimates only were comparable to those based on raw estimates.

Since endometriosis is an estrogen-dependent condition, the inverse association between smoking and endometriosis found in some studies has generally been attributed to the antiestrogenic effect of tobacco [83]. Some authors have suggested that estradiol might modulate the mediators of immune system molecules or those involved in tissue cell adhesion and invasion [84, 85]. Moreover, a favorable effect of smoking has been observed in other benign and malignant estrogen-related diseases, such as endometrial cancer [86], and fibroids [87]. The antiestrogenic effect of smoking on these conditions could support a protective effect of smoking on endometriosis. Indeed, earlier studies tended to support some inverse association, which however declined over time, and accumulating evidence suggests the presence of some false positive findings in earlier studies [88]. Furthermore, tobacco smoking has been associated with female infertility [89], and thus the interpretation of the relation between smoking and endometriosis may be influenced by the role of infertility.

Despite the high prevalence of this condition, the epidemiology of endometriosis still needs to be elucidated, for several reasons. Endometriosis is a complex condition in which a genetic contribution and environmental factors seem to be involved [90]. Further, it is a disease characterized by a still poorly defined phenotype. The disease stage depends on the type (cysts, implants, nodules), location (ovary, peritoneum, bladder, ureter, etc.), appearance and depth of invasion of the lesions, that can vary greatly among patients. The clinical presentation can be so variable and the lesions of such diverse morphology that none of the pathogenetic models proposed (retrograde menstruation, coelomic metaplasia, embryological origin) can fully explain the various aspects of endometriosis, and none has been recognized as an ultimately valid explanatory model for all the different forms and manifestations of the disease [90]. Moreover, an invasive procedure is needed to diagnose it [90, 91]. Furthermore, published studies differ in the case and control selection and population definition, depending on the choices to consider fertile or infertile cases, and healthy controls or patients with conditions other than endometriosis. Despite these possible sources of variation~~s~~, the consistency of results observed weighs against any relevant role of tobacco on endometriosis.

In conclusion, the present meta-analysis ~~gives no support to the hypothesis of~~ failed to identify an association between tobacco smoking and endometriosis. However, given the possible limitations of the present study, further studies are needed to evaluate in deep the relationship and the potential effect of smoking on different type of endometriosis.

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Contributors

FP conceived the idea and planned the research. FB and SC performed the statistical analysis. FB, FC, ER, VC retrieved data. FP, FB, PV and CLV wrote the entire draft of the article and all subsequent drafts after critical review by all co-authors. All co-authors gave significant input in the preparation of the article and the analysis. FP is the guarantor for the article.

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FIGURE LEGENDS

Figure 1 – Flow chart of the selection of studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

Figure 2 – Study-specific and summary relative risks (RR) of endometriosis for ever smokers versus non smokers.

CI: confidence interval.

Figure 3 – Study-specific and summary relative risks (RR) of endometriosis for current (A) and former smokers (B) versus non smokers.

CI: confidence interval.

Figure 4 – Study-specific and summary relative risks (RR) of endometriosis for moderate (A) and heavy (B) current smokers versus non smokers.

CI: confidence interval.

Figure 5 – Funnel-plot of studies on tobacco smoking and risk of endometriosis.

RR: relative risk for ever smokers versus non smokers; CI: confidence interval; s.e.: standard error.

Figure 6 - Cumulative meta-analysis of studies on tobacco smoking and risk of endometriosis.

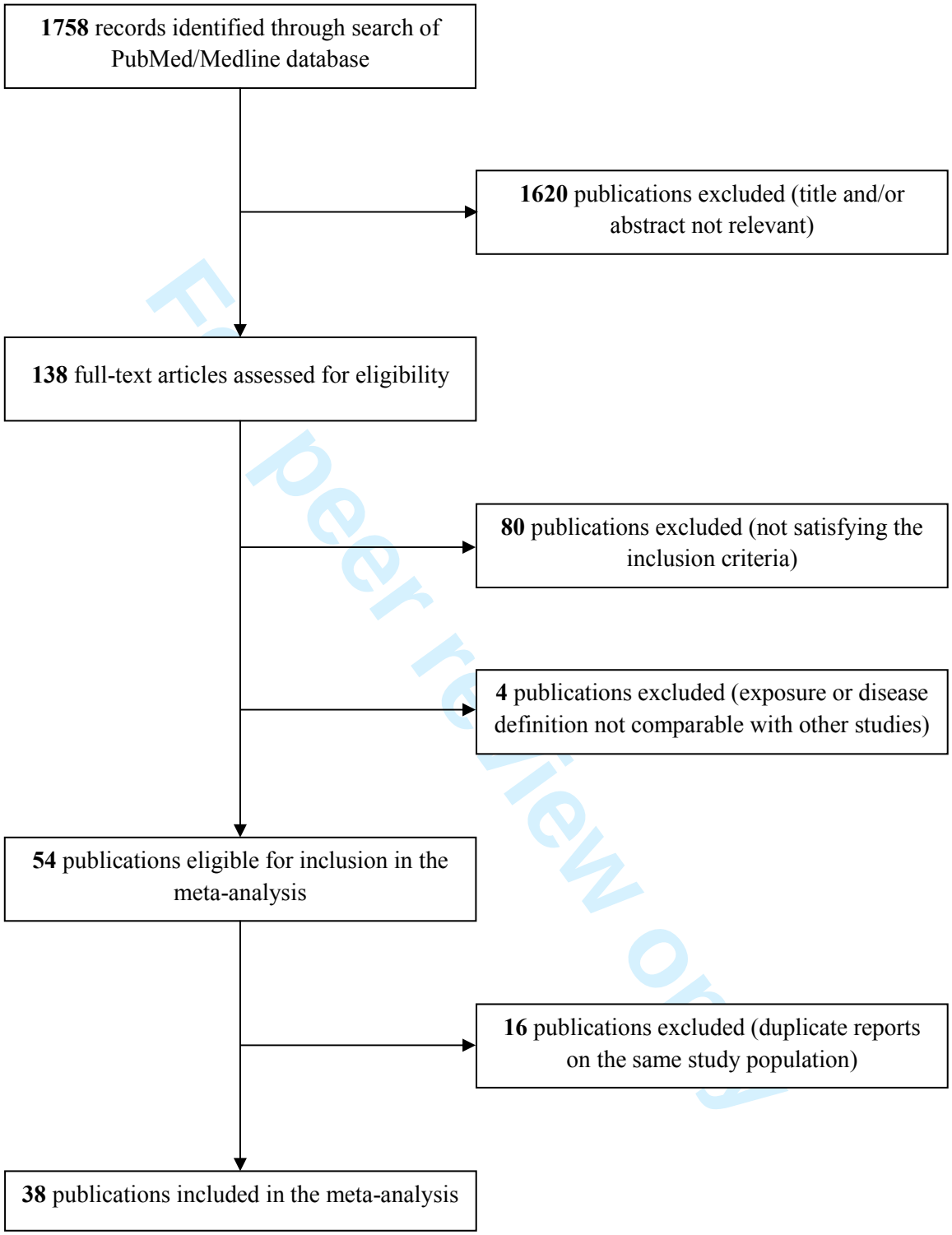
RR: relative risk for ~~coffee consumption versus no consumption~~ever smokers versus non smokers;

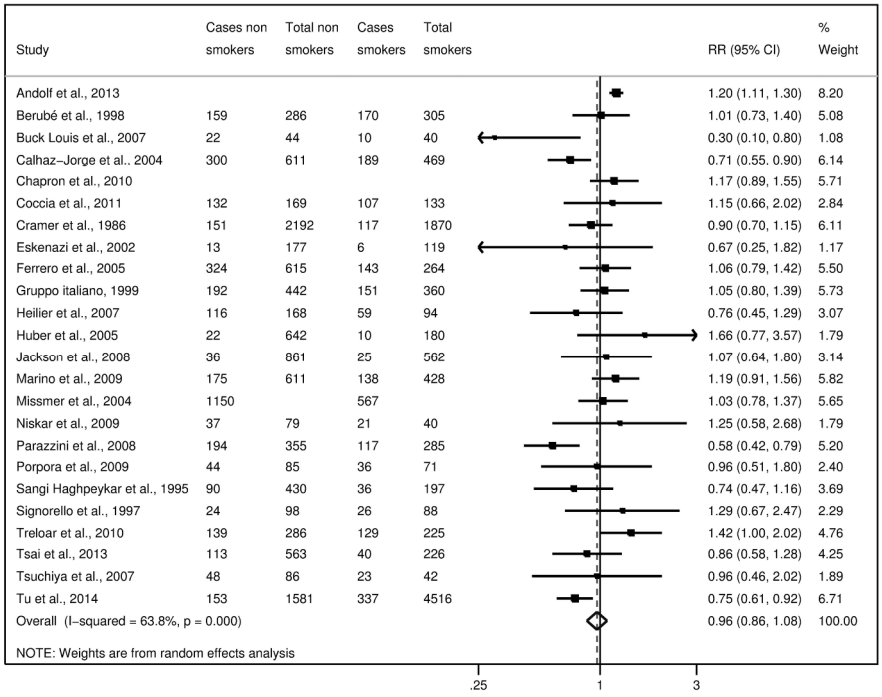
CI: confidence interval.

Appendix

The PubMed search was performed using the following search terms: "tobacco"[MeSH Terms] OR tobacco[Text Word] OR "smoking"[MeSH Terms] OR smoking[Text Word] OR cigarette[All Fields] OR risk factor OR epidemiology AND endometriosis. The search was limited to papers on Humans, written in English.

Figure 1





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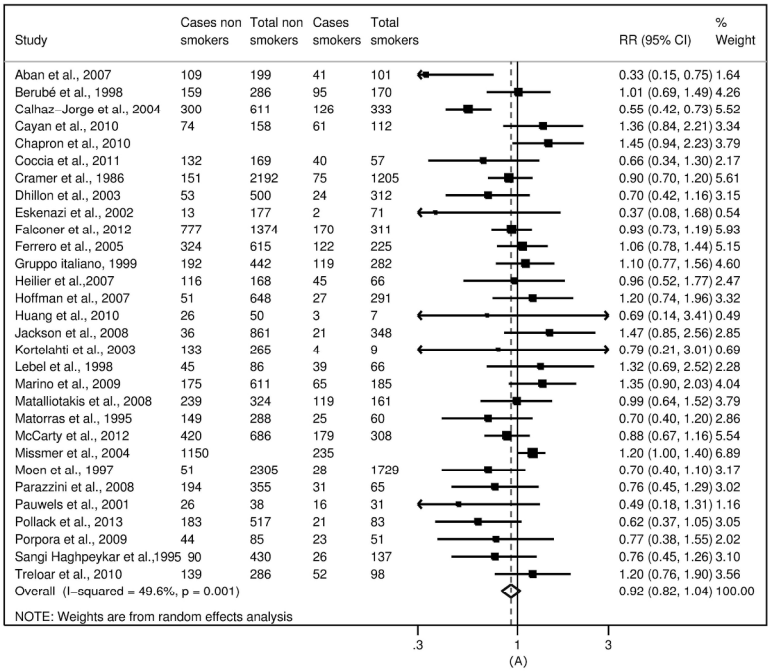

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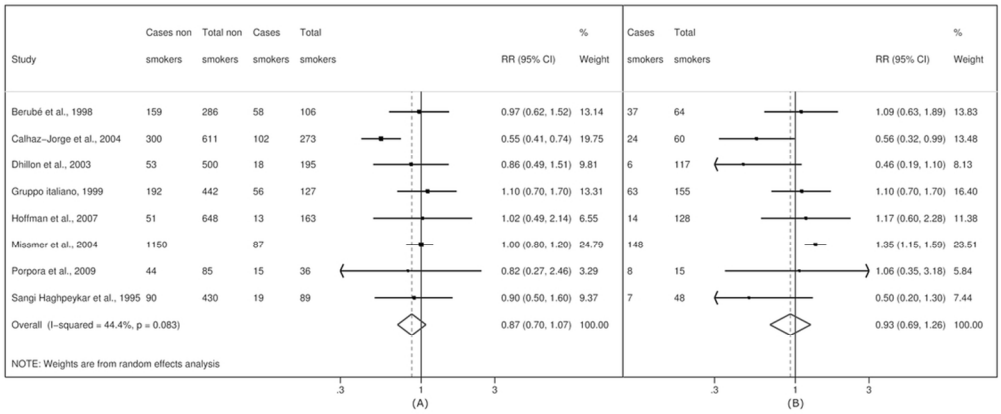

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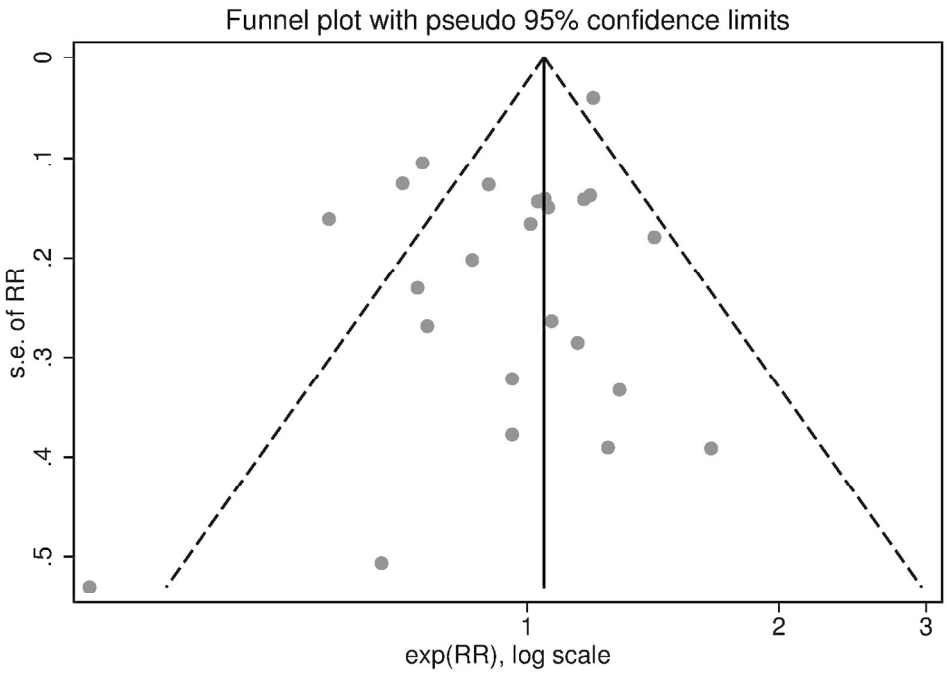

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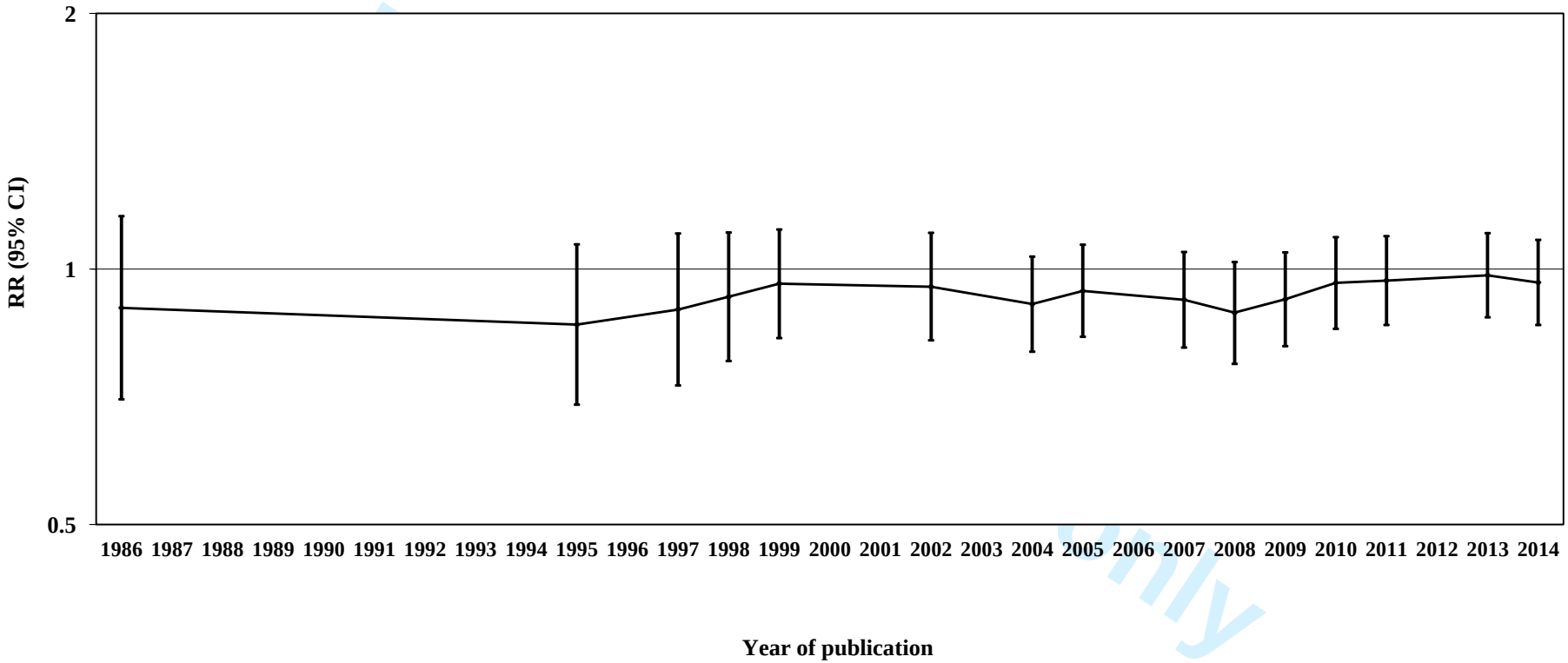

Supplementary file

Table 1 – Main characteristics of the studies on tobacco smoking and risk of endometriosis included in the meta-analysis.

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Aban et al., 2007 [6]	Turkey	Case-control	Women with endometriosis (surgically and histologically confirmed)	Women without endometriosis (surgically confirmed) undergoing tubal ligation, infertility workup, or ovarian cystis workup	150/150	mean 33.06 ± 8.67 for cases and 34.04 ± 9.68 for controls	Never, current smoker	Body mass index, age at menarche, education, socioeconomic status, cycle length, duration of bleeding	7
Andolf et al., 2013 [49]	Sweden	Cohort	Women who delivered their first born, with endometriosis (identified in the Swedish Patient Register)	Women who delivered their first born, without endometriosis (identified in the Swedish Patient Register)	3110/705980	<55	Ever smoker	Caesarean section, maternal age, body mass index, years of involuntary childlessness	6
Berubé et al., 1998 [8]	Canada	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	329/262	20-39	Never, former, current smoker (<20, ≥20 cigarettes/day)	-	5
Buck Louis et al., 2007 [50]	USA	Cohort	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis	32/52	18-40	Never, ever smoker	Age	4

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Calhaz-Jorge et al., 2004 [5]	Portugal	Cohort	Infertile women with endometriosis (laparoscopically confirmed); separate groups of grade I-II and grade III/IV endometriosis	Infertile women without endometriosis (laparoscopically confirmed)	488/591	mean 30.9 ± 3.9 for AFS grade I/II, 30.7 ± 4.0 for ASF grade III/IV and 30.9 ± 4.2 for controls	Never, former, current smoker (1-10, 11-20, >20 cigarettes/day)	Ethnicity, dysmenorrhoea, chronic pelvic pain, cycle regularity, body mass index, previous pregnancies, ever OC use	4
Cayan et al., 2010 [51]	Turkey	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	135/135	mean 39.36 ± 8.88 for cases and 41.6 ± 8.92 for controls	Non smoker, smoker	-	4
Chapron et al., 2010 [9]	France	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	411/567	<42 years	Ever, former, current smoker	Age, ethnicity, gravidity, parity, infertility, body mass index	7
Coccia et al., 2011 [52]	Italy	Cohort	Women with endometriosis (laparoscopically confirmed) Separate groups of monolateral and bilateral endometriosis	Women without endometriosis (laparoscopically confirmed)	239/63	mean 32.6 ± 5.6	Never, former, current smoker	-	5

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Cramer et al., 1986 [7]	USA	Case-control	Infertile women with endometriosis	Women admitted to hospital for delivery	268/3794	NA	Never, former, current smoker	Center, age, education, religion, years since menarche, menstrual pain, cycle length, weight, height, exercise	4
Dhillon et al., 2003 [53]	USA	Case-control	Women with cystic ovarian endometriosis (endometrioma)	Women receiving care from the same health maintenance organization	77/735	18-39	Non smoker, smoker (≤0.5, 0.5-1, ≥1 packs/day)	-	3
Eskenazi et al., 2002 [54]	Italy	Cohort	Women ≤30 yrs in 1976 with stored sera resident near Seveso in1976, with endometriosis (confirmed through laparoscopy, laparotomy or ultrasound)	Women ≤30 yrs in 1976 with stored sera resident near Seveso in 1976	19/277	≥20	Never, former, current smoker	-	6
Falconer et al., 2012 [55]	Belgium	Case-control	Women with endometriosis who underwent laparoscopy for subfertility	Women without endometriosis who underwent laparoscopy for subfertility	947/738	mean 31.5 ± 4.7 for cases and 32.1 ± 5.0 for controls	Current smoker	-	4

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Ferrero et al., 2005 [56]	Italy	Case-control	Women of reproductive age undergoing surgery because of uterine myomas, ovarian cysts, pelvic pain, dysmenhorrea, or infertility with endometriosis (histologically confirmed)	Women of reproductive age undergoing surgery because of uterine myomas, ovarian cysts, pelvic pain, dysmenhorrea, or infertility without endometriosis (histologically confirmed)	467/412	mean 34.3 ± 6.0 for cases and 34.5 ± 4.9 for controls	Never, former, current smoker	-	4
Gruppo Italiano per lo Studio dell'endometriosi, 1999 [10]	Italy	Case-control	Women with infertility or pelvic pain with endometriosis (laparoscopically confirmed); separate groups of pelvic pain and infertility	Women with infertility or pelvic pain without endometriosis (laparoscopically confirmed); separate groups of pelvic pain and infertility	345/472	18-43	Never, former, current smoker (<10, ≥10 cigarettes/day)	Age, parity, center, education, marital status	7
Heilier et al., 2007 [57]	Belgium	Case-control	Women with peritoneal endometriosis or deep endometriotic nodules (surgically confirmed); separate groups of endometriosis and deep endometriotic nodules	Women who consulted the same gynecologists of cases, with no clinical evidence of endometriosis	88+88/88	21-50	Never, former, current smoker	-	3
Hoffman et al., 2007 [58]	USA	Cohort	Women enrolled in the Michigan Polybrominated Biphenyls cohort, with self-reported endometriosis	Women enrolled in the Michigan Polybrominated Biphenyls cohort, without endometriosis	79/864	mean 45 ± 14.4	Non, current smoker (1-15, >15 cigarettes/day)	.	2

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Huang al., 2010 [59]	Taiwan	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis, adenomyosis and leiomyomas (laparoscopically confirmed)	28/29	mean 34.3 ±7.5 for cases and 36.2 ± 9.0 for controls	Current smoker	-	5
Huber et al., 2005 [60]	Austria	Case-control	Women with endometriosis (surgically and histologically confirmed)	Healthy women without endometriosis (based on personal interview)	32/790	mean 52.3 ± 5.4 for cases and 34.6 ±7.0 for controls	Ever smoker	-	5
Jackson et al., 2008 [61]	USA (NHANES study)	Case-control	Women with self-reported diagnosis of endometriosis	Women without self-reported diagnosis of endometriosis	61/1362	20-49	Never, former, current smoker	-	2
Kortelahti et al., 2003 [62]	Finland	Case-control	Women with endometriosis (histologically confirmed)	Women who underwent laparoscopy for tubal sterilization, and women who underwent in vitro fertilization for reasons other than endometriosis	137/137	mean 31.2 ± 5.1 for cases and 34.0 ± 4.6 for controls	Current smoker	-	3
Lebel et al., 1998 [63]	Canada	Case-control	Premenopausal women with endometriosis (laparoscopically confirmed)	Premenopausal women without endometriosis (laparoscopically confirmed)	86/70	18-50	Current non smoker	-	5

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Marino et al., 2009 [64]	USA	Case-control	Women enrolled in a health maintenance organization with surgically confirmed endometriosis	Women enrolled in a health maintenance organization without endometriosis	313/727	18-49	Never, former, current smoker	-	5
Matalliotakis et al., 2008 [12]	USA	Case-control	Women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis undergoing laparoscopy	535/200	15-56	Current smoker	-	5
Matorras et al., 1995 [3]	Spain	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	174/174	mean 29.49 ± 3.41 for cases and 29.58 ± 3.66 for controls	Current smoker	-	4
McCarty et al., 2012 [65]	USA	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (laparoscopically confirmed)	796/501	≥18	Never smoker	-	5
Missmer et al., 2004 [13]	USA	Cohort (Nurese Health Study II)	Women with self-reported endometriosis	Women aged without self-reported endometriosis	1721/88344	25-52	Never, former, current smoker (1-14, 15-24, 25-34, ≥35 cigarettes/day)	Age, calendar time, race, parity, body mass index at 18, alcohol drinking	5
Moen et al., 1997 [66]	Norway	Case-control	Women with self-reported endometriosis	Women aged without self-reported endometriosis	79/3955	40-42	Current smoker	-	2
Niskar et al., 2009 [67]	USA	Case-control	Nulliparous women seeking reproductive assistance with endometriosis (laparoscopically confirmed)	Nulliparous women seeking reproductive assistance without endometriosis	60/64	20-45	Ever smoker	-	4

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Parazzini et al., 2008 [68]	Italy	Case-control	Women with deep endometriosis or pelvic and ovarian endometriosis (laparoscopically confirmed); separate groups of deep endometriosis and pelvic and ovarian endometriosis	Women without endometriosis admitted to hospital for acute non-gynecological, non-hormonal, non-neoplastic conditions, participating as controls in a case-control study on female genital neoplasms	181 + 162/329	20-55	Never, former, current	-	5
Pauwels et al., 2001 [69]	Belgium	Case-control	Infertile women with endometriosis (laparoscopically confirmed)	Infertile women without endometriosis (laparoscopically confirmed)	42/27	24-42	Non smokers	-	5
Pollack et al., 2013 [70]	USA	Cohort	Women with endometriosis (confirmed through laparoscopy or magnetic resonance imaging)	Women without endometriosis (confirmed through laparoscopy or magnetic resonance imaging)	204/396	18-44	Current smoker	-	5
Porpora et al., 2009 [71]	Italy	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis who underwent laparoscopy for benign gynecological conditions (unrelated to infertility)	80/78	18-45	Never, former, current smokers (1-9, 10-19, ≥20 cigarettes/day)	-	4

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Sangi-Haghpeykar et al., 1995 [72]	USA	Case-control	Women undergoing laparoscopic tubal sterilization with endometriosis	Women undergoing laparoscopic tubal sterilization without endometriosis	126/504	NA	Never, former, current smoker (< 1 pack/day, ≥ 1 pack/day)	Age, number of live births	5
Signorello et al., 1997 [14]	USA	Case-control	Women with infertility-associated endometriosis (laparoscopically confirmed)	fertile and infertile women both without endometriosis (laparoscopically confirmed); separate groups of fertile and infertile controls	50/89 + 47	23-44	Never, ever smoker	-	4
Treloar et al., 2010 [73]	Australia	Case-control	Women with endometriosis (surgically confirmed) with no first degree relative with endometriosis	Same-sex female twin pairs enrolled with the Australian Twin Registry, without endometriosis (self-reported)	268/244	18-55	Never, former, current smoker	-	3
Tsai et al., 2013 [74]	Taiwan	Case-control	Women with endometriosis (laparoscopically confirmed)	Women without endometriosis (confirmed through ultrasonography)	153/636	mean 40.3 ± 4.9 for cases	Ever smoker	-	3
Tsuchiya et al., 2007 [75]	Japan	Case-control	Women who had not given birth or lactate, with endometriosis (laparoscopically confirmed); separate groups of stage I/II and stage III/IV endometriosis	Women who had not given birth or lactate without endometriosis (laparoscopically confirmed)	79/59	20-45	Never, ever smoker	-	5

Study	Country	Study design	Cases	Controls	Sample size cases/controls	Age (years)	Smoking habit	Confounding factors	Quality score
Tu et al., 2014 [76]	Australia	Cohort	Women with endometriosis (self-reported diagnosis by a clinician)	Women without endometriosis	490/5607	18-23	Never, ever smoked (less than daily for 6 months, daily for 6 months)	-	4

[1]Based on the Newcastle-Ottawa Score [22]. NA: not available; NHANES: National Health and Nutrition Examination Survey; OC: oral contraceptive

Appendix

The PubMed search was performed using the following search terms: "tobacco"[MeSH Terms] OR tobacco[Text Word] OR "smoking"[MeSH Terms] OR smoking[Text Word] OR cigarette[All Fields] OR risk factor OR epidemiology AND endometriosis. The search was limited to papers on Humans, written in English.

MOOSE Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies*

Title	Identify the study as a meta-analysis (or systematic review)
Abstract	Use the journal's structured format
Introduction	**Present** • The clinical problem • The hypothesis • A statement of objectives that includes the study population, the condition of interest, the exposure or intervention, and the outcome(s) considered
Sources	**Describe** • Qualifications of searchers (eg, librarians and investigators) • Search strategy, including time period included in the synthesis and keywords • Effort to include all available studies, including contact with authors • Databases and registries searched • Search software used, name and version, including special features used (eg, explosion) • Use of hand searching (eg, reference lists of obtained articles) • List of citations located and those excluded, including justification • Method of addressing articles published in languages other than English • Method of handling abstracts and unpublished studies • Description of any contact with authors
Study Selection	**Describe** • Types of study designs considered • Relevance or appropriateness of studies gathered for assessing the hypothesis to be tested • Rationale for the selection and coding of data (eg, sound clinical principles or convenience) • Documentation of how data were classified and coded (eg, multiple raters, blinding, and interrater reliability) • Assessment of confounding (eg, comparability of cases and controls in studies where appropriate) • Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results • Assessment of heterogeneity • Statistical methods (eg, complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated
Results	**Present** • A graph summarizing individual study estimates and the overall estimate • A table giving descriptive information for each included study • Results of sensitivity testing (eg, subgroup analysis) • Indication of statistical uncertainty of findings
Discussion	**Discuss** • Strengths and weaknesses • Potential biases in the review process (eg, publication bias) • Justification for exclusion (eg, exclusion of non–English-language citations) • Assessment of quality of included studies • Consideration of alternative explanations for observed results • Generalization of the conclusions (ie, appropriate for the data presented and within the domain of the literature review) • Guidelines for future research • Disclosure of funding source

*Modified from Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008–12.