Fibroids and natural fertility: a systematic review and meta-analysis

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ABSTRACT

Research question: Do fibroids interfere with natural conception?

Design: A systematic review and meta-analysis was performed. We were interested in studies comparing fertile and infertile women and investigating whether the presence of fibroids was a risk factor as well as studies comparing women with and without fibroids and evaluating whether the frequency of infertility differed between the two groups.

Results: Seven out of the eleven selected studies were poorly informative because they did not aim at disentangle whether or not fibroids could cause infertility but, instead, whether a history of infertility could be a risk factor for fibroids. A meta-analysis of the four remaining studies that concomitantly evaluated the presence of fibroids and infertility studies highlighted a common OR of fibroids in subfertile women of 3.54 (95%CI: 1.55-8.11). However, when focusing on the two most informative studies, i.e. the studies that compared time to pregnancy in women with and without fibroids, the common OR was 1.93 (95%CI: 0.89-4.18).

Conclusions: The relation between fibroids and infertility has been insufficiently investigated Epidemiological studies suggest but do not demonstrate that fibroids may interfere with natural fertility. Given the high prevalence of these lesions in women seeking pregnancy, further evidence is pressingly needed.

KEY WORDS: fibroid; myoma; leiomyoma; fertility; infertility

INTRODUCTION

There is a general consensus that fibroids can cause subfertility (Somigliana *et al*., 2007; Horne and Critchley, 2007; Pier and Bates, 2015; Zepiridis *et al*., 2016). Several pathogenetic findings support this conclusion. Fibroids can disrupt the physiological miometrial motility interfering with both spermatozoa progression and embryo implantation (Nishino *et al*., 2005; Orisaka *et al*., 2007; Yoshino *et al*., 2010). They can subvert the pelvic anatomy thus perturbing the function of the tubes (Somigliana *et al*., 2007). Finally, submucosal but possibly also intramural lesions can create a deleterious endometrial inflammatory milieu that can affect both spermatozoa migration and embryo implantation (Ikhena and Bulun, 2018; Munro, 2019).

On the other hand, despite this body of evidence, epidemiological evidence linking fibroids and infertility is still controversial. There are some methodological difficulties that can explain this lack of conclusive evidence (Table 1). Infertile women are generally nulliparous and parity displays a remarkable protective effect on the development of fibroids (Wise and Laughlin-Tommaso, 2016; Stewart *et al*., 2017). In fact, infertile women may be exposed to a higher risk of fibroids just because they have no children. Fibroids prevalence increases with women age (Wise and Laughlin-Tommaso, 2016; Stewart *et al*., 2017) while fertility declines with woman age (Schmidt *et al*., 2012; ESHRE Capri Workshop Group, 2017). This intricate relation is particularly detrimental considering the modern trend in Western countries to delay motherhood to the late thirties if not the forties (Mills *et al*., 2011), i.e. at an age when fibroids become more common (Giuliani *et al*., 2020). Moreover, fibroids are associated with gynecological conditions that can cause infertility such as in particular endometriosis (Hemmings *et al*., 2004; Uimari *et al*., 2011; Capezzuoli *et al*., 2020). Again, it may be difficult to discern whether fibroids have a direct detrimental impact or, conversely, whether the association with infertility is mediated by the concomitant presence of conditions that can interfere with fertility. In addition, study designs are complicated by the fact that fibroids are a heterogeneous condition with huge differences among affected women in terms of number, dimension and location of lesions (Munro *et al*., 2011; Stewart *et al*., 2016). The relative effect of each type of lesion on fertility may differ and the combination of different lesions within the same subject may ultimately have a very peculiar effect. Finally, the majority of fibroids do not cause pain or cycle disturbances (Lumsden *et al*., 2015; Stewart *et al*., 2016), most are undetected if women do not undergo regular screening ultrasounds and their natural history is long and unpredictable (Mavrelos *et al*., 2011; Armbrust *et al.*, 2018). As a consequence, a main challenge in studies investigating the relation between infertility and fibroids is properly handling temporality. Were fibroids already present when the studied woman initiated to seek for pregnancy? Did they modify over time during the period of pregnancy seeking?

In this scenario, designing studies that effectively investigate the causal relation between fibroids and infertility is inevitably challenging.

THE MODEL OF IVF

In vitro fertilization (IVF) has been considered a valuable model to provide information on the impact of fibroids on infertility. It is also methodologically straightforward. Studies mainly consisted in recruiting women undergoing IVF and evaluating the chances of pregnancy in women with and without fibroids. Multivariate analyses can also be drawn to control for confounders such as age, ovarian response and indication to IVF. To note, even if prospective recruitment could provide more reliable evidence, retrospective studies could also be informative, in particular in Centers that systematically perform accurate sonography prior to initiate IVF cycles. Not surprisingly, a plethora of studies using this study design has been published over the last two decades and several metanalyses became available over the years (Pritts 2001; Benecke *et al*., 2005; Somigliana *et al*., 2007; Pritts *et al.*, 2009; Sunkara *et al*., 2010; Metwally *et al*., 2011; Guven *et al*., 2013; Wang *et al*., 2018; Rikhraj *et al*., 2020). Whereas the first ones included all type of fibroids, the later ones focused on intramural lesions because of the robustness of the early emerged messages showing that submucosal lesions were detrimental while subserosal lesions were not. More specifically, since the first meta-analysis of Pritts in 2001 that clearly showed a detrimental effect of submucosal lesions, no significant additional contributions were published. The last meta-analyses formerly reporting on this type of lesions was published in 2009 and showed a Relative Risk (RR) of pregnancy rate and ongoing/delivery rate of 0.36 (95%CI: 0.18-0.74) and 0.32 (95%CI: 0.12-0.85), respectively (Pritts *et al*., 2009). Considering subserosal lesions, the latest meta-analyses specifically reporting data for these lesions showed an Odds Ratio (OR) of clinical pregnancy and delivery rate of 1.2 (95%CI: 0.8-1.7) and 1.0 (95%CI: 0.7-1.5), respectively (Somigliana *et al*., 2007).

Conversely, the impact of intramural lesions remains a matter of concern and specific papers and adjourned meta-analyses on these lesions have continued to be published over the years (Sunkara *et al*., 2010; Metwally *et al*., 2011; Guven *et al*., 2013; Wang *et al*., 2018; Rikhraj *et al*., 2020). The latest available meta-analyses published in 2020 showed an OR of clinical pregnancy and live birth of 0.68 (95%CI: 0.56-0.83) and 0.56 (95%CI: 0.46-0.69), respectively (Rikhraj *et al*., 2020). An additional very recent study not included in the meta-analysis and investigating the role of intramural fibroids reported data that were overall in line with these findings (Bai *et al*., 2020).

IVF is an excellent model. However, it cannot be considered fully satisfactory, in particular if one aims at disentangling whether or not fibroids impair natural fertility. Of utmost relevance here is that the IVF model ignores some of the potentially detrimental mechanisms of fibroids and, in particular, the possible detrimental effects on sperm transit and tubal function. Moreover, evidence from IVF does not disentangle whether the interference is partial or complete. Available data for IVF are provided by single cycle studies that compared the rate of success in women with and without fibroids. They cannot discern whether the impairment is due to a relative reduction that could be overcome by just increasing the number of cycles or, conversely, whether the reduction has to be intended as an increase in the proportion of women who will never obtain a pregnancy. If the former is true, a reduction in pregnancy rate in IVF will not translate into a significant impairment of natural fertility (just an increase in time to pregnancy) while, in the latter situation, one would have to conclude for a definite interference with natural fertility. Last, but not least, we cannot exclude that IVF could be an effective therapy for fibroid-related infertility. If so, one could speculate that the absence of any effect of subserosal lesions in IVF settings may be interpreted as a demonstration of a total capacity of the procedure to restore normality. Furthermore, for intramural lesions, the observed reduced chance of success could be an under-estimation of the detrimental effects in natural conditions.

INTERVENTIONAL STUDIES

The demonstration that treatments of fibroid could improve both natural or IVF mediated pregnancy rate would indirectly support a detrimental effect of these lesions. However, information from this type of evidence is very limited.

Considering submucosal lesions, there is a general agreement on the benefits of hysteroscopic myomectomy for fibroids typo 0 and 1 (Munro *et al*., 2011), i.e. for those that are totally or mostly intra-cavitary (Zepiridis *et al*., 2016; Somigliana *et al*., 2007). However, it has to be recognized that scientific evidence is not robust. According to the most recent Cochrane meta-analyses on the argument (Bosteels *et al*., 2018; Metwally *et al*., 2020), only one low quality Randomized Controlled Trial (RCT) is available (Casini *et al*., 2006). It showed that removal of submucosal or submucosal/intramural fibroids was associated to an OR of clinical pregnancy of 2.44 (95%CI: 0.91-6.17) (Bosteels *et al*., 2018). Data on live birth was lacking, while the OR for miscarriage resulted 1.54 (95%CI: 0.47-5.00).

The scenario is even less comforting for non-submucosal lesions. Evidence from interventional studies is extremely modest and mostly consists in case series (Farquhar, 2009; Metwally *et al.*, 2020). The recent Cochrane meta-analysis (Metwally *et al.*, 2020) actually identified only one small and low quality RCT (Casini *et al.*, 2006), the same study that was selected for submucosal lesions. The ORs of clinical pregnancy for operated compared to non-operated women carrying intramural and intramural/subserosal lesions were 1.88 (95%CI: 0.57-6.14) and 2.00 (95%CI: 0.40-10.09), respectively (Metwally *et al.*, 2020). The OR of miscarriage for both intramural and intramural/subserosal was 2.00 (95%CI: 0.32-12.33). Live birth rate was not reported (Metwally *et al.*, 2020).

Some comparative non randomized studies were also published to test the potential benefits of surgery for both natural pregnancy (Bulletti *et al.*, 1999) and IVF-mediated pregnancy (Bulletti *et al.*, 2004). They showed a significant improvement of the chances of pregnancy after surgery. However, this evidence should be interpreted with caution because the studies were not randomized. Moreover, it has to be underlined that only women with large fibroids (> 4 cm in diameter) were included and potential inferences should thus be limited to these advanced conditions.

AIM OF THE STUDY

Currently available evidence supports a detrimental effect of submucosal fibroid and a possible negative effect of intramural lesions on implantation in IVF. Several systematic reviews and meta-analyses have addressed these aspects. Conversely, to the best of our knowledge, there is no systematic review aimed at clarifying the impact of fibroids on natural fertility. In our opinion, this information represents an important clinical need. Physicians are frequently faced to infertile women with fibroids and are called to provide counseling and decide management strategies. For this reason, we deemed important reviewing in depth the current available literature on the possible impact of fibroids on natural fertility.

MATERIALS AND METHODS

This review was restricted to published research articles that reported on the impact of fibroids on natural fertility. The main outcome was achievement of a natural pregnancy. The research excluded evidence referring to pregnancies exclusively obtained with IVF. We were interested in studies comparing fertile and infertile women and investigating whether the presence of fibroids was a risk factor as well as studies comparing women with and without fibroids and evaluating whether the frequency of infertility differed between the two groups. The study was registered in PROSPERO (International prospective register of systematic reviews) in March 17th, 2020 (ID: CRD42020178750).

Literature overview was conducted according to the PRISMA guidelines for systematic reviews (Moher *et al*., 2009). As published de-identified data were used, this study was exempt from institutional review board approval. The primary search was conducted with Medline, including the time period from January 1980 to May 2020 and using the following search strings: (fibroid OR myoma OR leiomyoma OR leiomyomata) AND (infertility OR fertility OR conception OR time to pregnancy OR reproductive history) AND (case control OR cohort OR cross-sectional). The research was re-checked with EMBASE using the same combination of terms. Data from case-control, cohort and cross-sectional studies could all be included. Conversely, case reports and case series were excluded. When study periods of studies performed in the same Institution overlapped, only the larger one was included. Publications not written in English were excluded. All pertinent articles were retrieved, and the relative reference lists checked to identify further publications. Moreover, the main review articles on fibroids published over the last 10 years were consulted and their reference lists searched for potential additional studies. No attempt was made to contact authors for incomplete information and to identify unpublished studies or abstracts submitted to national or international conferences. All this selection process was conducted independently by three of the authors (ES, LL and VB) and discordances were solved by discussion.

Quality of the case-control or cohort studies was evaluated using the Newcastle Ottawa Scale (NOS). This scale gives up to 9 stars to each study and classify them as low quality (0–4 stars), moderate quality (5-6 stars) and high quality (7-9 stars) (Wells *et al*., 2018).

From each selected article, we extracted the information on study characteristics, type of fibroid, history of infertility and pregnancy rate. Studies were grouped for the meta-analysis only if they had the same study design. Results are expressed as Incidence Rate Ratio (IRR), RR or OR with 95%CI. The calculated and extracted effect estimates were combined in meta-analyses using the generic inverse variance method, with the DerSimonian and Laird random-effects model (DerSimonian and Kacker, 2007; Busnelli *et al*., 2020). The effect estimates were combined as they were extrapolated from the studies and no attempts were made to convert one effect estimate into another. To compare data from different studies, definitions and cut-offs of risk factors were harmonized between studies whenever possible. The inconsistency of studies' results was measured using Cochrane Q and the I^2 statistics (Higgins *et al*., 2003). Negative values of I^2 are set equal to 0 so that I^2 lies between 0 and 100%. According to the *Cochrane Handbook for Systematic Reviews of Intervention* an I^2 value of 0% indicates no observed heterogeneity, whereas I^2 values from 30% to 60% may represent moderate heterogeneity, I^2 values from 50% to 90% may indicate substantial heterogeneity and I^2 values from 75% to 100% express considerable heterogeneity (Deeks *et al*., 2018; Higgins *et al*., 2003; Busnelli *et al*., 2019). Data was analyzed using the Review Manager 5.3 (Englewood, USA).

RESULTS

The initial literature search returned 1,058 articles. The selection process that followed is depicted in Figure 1. Eleven studies were ultimately selected (Parazzini *et al*., 1996; Marshall *et al*., 1998; Bulletti *et al*., 1999; Faerstein *et al*., 2001; Wise *et al*., 2004 ; Wellons *et al*., 2008 ; Templeman *et al*., 2009 ; Johnson *et al*., 2012; Yasui *et al*., 2018 ; Karlsen *et al*., 2020; Egbe *et al*., 2020). Their main characteristics are shown in Table 2. The majority of studies (n=6) was performed in the US. Six studies evaluated a history of infertility in women with and without fibroids. Four investigated the presence of fibroids in women with and without infertility: two of them focused on a prolonged duration of pregnancy seeking (subfertility) rather than frank infertility (Johnson *et al*., 2012; Karlsen *et al*., 2020). One study evaluated natural pregnancy in infertile women with and without fibroids (Bulletti *et al*., 1999). Four studies were cohort studies, three were case-control studies and four were cross-sectional. Recruitment was prospective in ten studies (Table 2).

Surgery or ultrasounds were the most common mean of diagnosis of fibroids (n=7). In two studies, the diagnosis was self-reported (but validated) (Marshall *et al*., 1998; Yasui *et al*., 2018) and in two studies the methodology was not explained (Karlsen *et al*., 2019; Egbe *et al*., 2020). None of the studies excluded women with submucosal lesions but data according to fibroid location was reported in only one of them (Johnson *et al*., 2012). Data on the possible concomitant causes of infertility (such as the presence of a male factor of infertility) was not reported in five studies, partly assessed in two studies and collected in the remaining three. In one study, the presence of identified causes of infertility was an exclusion criterion (Bulletti *et al*., 1999). In none of the selected studies, analyses were drawn according to the possible cause of infertility.

The quality of the evidence is generally elevated. It could be assessed in 7 studies and in 6 of them the Newcastle-Ottawa Scale was ≥ 6 (Wells *et al*., 2018). The quality was low (score of 4) in one study (Egbe *et al*., 2020).

In four studies, infertility and fibroids were concomitant (Bulletti *et al*., 1999; Johnson *et al*., 2012; Karlsen *et al*., 2020; Egbe *et al*., 2020). The temporal relation between infertility and fibroids, i.e. whether they did or did not overlap could not be assessed in the remaining seven studies.

META-ANALYSES

The main results documented in the eleven included studies are summarized in Table 3. Eight of them supported a possible association.

Given the disparity of the study designs, data could not be combined in a unique meta-analysis. At first, we performed three separate analyses (Figure 2).

The first focused on a history of infertility in the three cohort studies comparing women with and without fibroids (Marshall *et al*., 1998; Wise *et al*., 2004; Templeman *et al*., 2009). The common IRR of fibroids in women with a history of infertility was 1.20 (95%CI: 0.98-1.47, $p=0.07$) (Figure 2, panel A).

The second focused on the six case-control or cross-sectional studies investigated the association between a history of infertility (or infertility) and fibroids (Parazzini *et al*., 1996; Bulletti *et al*., 1999; Faerstein *et al*., 2001; Wellons *et al*., 2008; Yasui *et al*., 2018; Egbe *et al*., 2020). The common OR of fibroids in women with a history of infertility was 2.18 (95%CI: 1.47-3.25, $p<0.001$) (Figure 2, panel B). Given the high heterogeneity and the outlier results reported by the low quality study from Egbe et al. (Egbe *et al*., 2020), we repeated the meta-analysis excluding that study. The heterogeneity remained high (I^2=76%). The common OR based on a random model was 1.85 (95%CI: 1.32-2.60, $p<0.001$).

The third meta-analysis grouped the two studies comparing the presence of fibroids in fertile and subfertile pregnant women (Johnson *et al*., 2012; Karlsen *et al*., 2019). The common OR of fibroids in subfertile women was 1.93 (95%CI: 0.89-4.18, p=0.10) (Figure 2, panel C).

Finally, we performed a meta-analysis grouping the four studies whose study design foresaw the recruitment of women with the concomitant presence of fibroids and infertility (Bulletti *et al*., 1999; Johnson *et al*., 2012; Karlsen *et al*., 2020; Egbe *et al*., 2020). The results are illustrated in Figure 3. The common OR of fibroids in subfertile women was 3.54 (95%CI: 1.55-8.11, p=0.003). When excluding the study from Egbe *et al*. (Egbe *et al*., 2020), the I^2 was 90% and the common OR was 2.64 (95%CI: 1.19-5.88, p=0.02).

COMMENT

Our study showed that the relation between fibroids and natural fertility has been poorly and inadequately investigated. Seven out of the eleven selected studies did not aim at disentangle whether or not fibroids could cause infertility but, instead, whether or not a history of infertility could be a risk factor for fibroids (Parazzini *et al*., 1996; Marshall *et al*., 1998; Faerstein *et al*., 2001; Wise *et al*., 2004; Wellons *et al*., 2008; Templeman *et al*., 2009; Yasui *et al*., 2018). Given this aim, the authors of these studies did not adequately handle the confounding effect of temporality. They did not collect information on the time passed between the infertility period and the development of fibroids, thus hampering any conclusion on the possible inverse causal relation. They provide information on the possible detrimental effect of infertility on fibroids development but not on the possible detrimental effects of fibroids on fertility. Given the aim of the present study, these studies should be considered non informative. On the other hand, there is no definite evidence to support our concern and, for this reason, we did not exclude these studies from our systematic review.

To overcome this limitation, we decided to perform a separate analysis for the remaining four studies (Bulletti *et al.*, 1999; Johnson *et al.*, 2012; Karlsen *et al.*, 2020; Egbe *et al.*, 2020). The common OR of fibroids in subfertile women was 3.54 (95%CI: 1.55-8.11). Even excluding the outlier findings of Egbe et al., the association remained significant (OR=2.64, 95%CI: 1.19-5.88). Even if a clear temporal relation cannot be discerned also in that studies, fibroids and fertility were at least concomitantly assessed. Specifically, Bulletti *et al.* evaluated the chances of natural pregnancy after surgery in three distinct groups of women with infertility or recurrent miscarriages: 1) women with fibroids who underwent excision of the lesions at the time of laparoscopy, 3) women with fibroids who performed only diagnostic laparoscopy, 3) women with unexplained infertility without fibroids who also performed only diagnostic laparoscopy. For the purpose of our review (investigating the impact of fibroids on natural conception, not the impact of miomectomy), we retrieved and compared data only from the two latter groups (Bulletti *et al.*, 1999). These authors, however, included only women with advanced conditions (at least one fibroid larger than 4 cm), thus hampering inferences to the whole population of women with fibroids. To note, the vast majority of included women (77%) had one of more fibroids with a mean diameter above 6 cm, a condition that is rarely asymptomatic. Pelvic pressure or discomfort is common in this situation. From a clinical perspective, this population is less interesting since surgery is generally indicated regardless of fertility status. In addition, Bulletti et al. did not present results separately for infertility and recurrent miscarriages, they included all fibroids locations (they did not exclude submucosal lesions), age and fibroids characteristics were not reported separately for the study groups and location relied exclusively on ultrasound (Bulletti *et al.*, 1999). The second study included in this analysis, the study from Egbe et al., compared the frequency of fibroids between women with and without bilateral tubal occlusion (Egbe *et al.*, 2020). The approach is original since it could highlight possible detrimental effects on tubal function but the quality of the study was not optimal and the findings should thus be interpreted with extreme caution. Finally, the remaining two studies (Johnson *et al.*, 2012; Karlsen *et al.*, 2019) merit particular attention because they referred to the concept of subfertility rather than infertility, i.e. they focused on a prolonged time to conceive rather than a definite state of incapacity to conceive. To note, this is in line with the modern vision of fertility disorders (Evers, 2002). A black and white vision (fertile / infertile) is currently considered simplistic and may not properly capture the problem. Fecundity, i.e. the chances of becoming pregnant per month, could better reflect the issue of infertility. On the other hand, these two studies were also not entirely satisfactory. Indeed, since they exclusively included pregnant women, most severe cases (those who are definitely infertile) were excluded. This could lead to an under-estimation of the magnitude of the association. In addition, even if one study used the threshold of 12 months that is commonly advocated to define a difficulty in conception (Karlsen *et al.*, 2019), the second study arbitrarily divided women based on a threshold of only 3 months (Johnson *et al.*, 2012). Information from this latest evidence is inevitably questionable. One may even argue that including that study in the meta-analysis was inappropriate. To note, despite the two studies were independently suggestive of an association with fibroids, the common OR calculated with the meta-analysis was not. Indeed, the high heterogeneity hampered the use of a fixed model and a statistical significance was not reached with the random model (OR=1.93, 95%CI: 0.89-4.18). In fact, excluding the study from Johnson et al that used a threshold of only 3 months would tip the balance in favor of a significant association.

Another main limitation of the available literature is the absence of subgroup information. Fibroids are a highly heterogeneous condition (Munro *et al.*, 2011) and we need more precise information on the possible detrimental effects of the total number of the lesions, their dimension and, most importantly, the location. It is worthwhile noting that all studies did not exclude submucosal lesions and only one reported data separately for this type of lesions (Johnson *et al.*, 2012). The inclusion of submucosal lesions is expected to enhance the association given the highly detrimental effect emerged in IVF studies. To note, from a clinical and research perspectives, these are the less interesting lesions because a relation with infertility is already given for granted and these lesions are frequently associated to metrorrhagia, a symptom that justifies *per se* surgical removal regardless of the concomitant fertility status.

FUTURE STUDIES

In this systematic review, we attempted to include any study that could be informative on the relation between fibroids and natural fertility. However, most studies were poorly significant, if not misleading. In addition, findings were frequently exposed to important confounders such as selection biases, diagnostic methods, failure to adjust for fibroids characteristics (size, location, number), temporal issues and definition of infertility. In particular, fibroids are an extremely heterogeneous condition and should not be aggregated into a unique diagnostic entity. This frequent experimental choice enhances the study power and simplified the analyses but dilutes the findings and cannot provide meaningful information for clinicians. Infertility would also merit a more precise definition. It is also a multifaceted condition and simplifying the definition to the time of pregnancy seeking without attempting to exclude cases with patent explanations (such as male factor or tubal diseases) is expected to temper or hide possible associations. Finally, studies comparing the frequency of a history of infertility in women with and without fibroids are difficult to interpret if it is not clarified whether or not fibroids were already present when the women initiated to seek for pregnancy (and none of the included studies reported on this aspect). Parity is a crucial protective factor against fibroids and it cannot be discerned whether a history of infertility is associated to fibroids just because infertile women have less or no children. Overall, in our opinion, there is the need to accept and handle the complexity of this argument.

In this regard, this overview of the literature offers the opportunity to discuss the possible study designs to be used for future investigations. We deem particularly interesting the use of prolonged time to pregnancy, as suggested by two of the most recent contributions (Johnson *et al*., 2012; Karlsen *et al*., 2019) and an additional abstract not yet published and therefore not included in this meta-analysis (Wise *et al*., 2015). Even if fully infertile women are excluded, this study design has the advantage of being relatively simple, avoiding the confounding effect of time and could consent to recruit a large sample size in a relatively short period of time. Most importantly, if one foresees an accurate and precise sonographic assessment, one could also retrieve valuable information on the independent role of the dimension, number and location of the lesions. Inferences would however to take into consideration the well-known detrimental effects of initial pregnancy on fibroid growth (in particular when speculating on the possible role of lesions' dimension) (Sarais *et al*., 2017).

An alternative study design could be the comparison of the frequency of fibroids between infertile women with unexplained infertility and those with severe male infertility. This study design could consent to overcome the problem of studies focusing on time to pregnancy that actually discard totally infertile (sterile) cases even if it is exposed to the possibility to dilute evidence because among controls (male factors), one may expect 10-15% of infertile women too. Finally, the study design proposed by Egbe et al (2020) consisting in comparing the frequency of fibroids between women with bilateral tubal occlusion and controls deserves consideration too. It can provide some valuable evidence on the possible detrimental role of fibroids on tubal function.

However, even if challenging, the most appropriate study design would obviously be a prospective longitudinal observation cohort study that recruits women before initiating pregnancy seeking. This study design requires important organizational efforts and relevant financial resources. All women would have to undergo a preliminary clinical and instrumental assessment. History of gynecological diseases such as endometriosis, PID and previous surgery for any gynecological disorders should be collected. Transvaginal ultrasound (or MRI) should be performed to identify, measure and locate all fibroids and to investigate the concomitant presence of gynecological disorders such as endometriosis that could interfere with fertility. A semen analysis should be obtained from the partner. Women with a current or past history of PID, those with submucosal fibroids and those whose partner displays severe semen alterations should be excluded. Conversely, excluding women with endometriosis or those with previous surgery for fibroids may be inappropriate because it may introduce selection biases. Information on these two points has to be properly handled at the time of data analyses. Finally, women would have to be monitored over time (every 6 months) to identify modifications in the number and dimension of the fibroids. Women should be included for at least one year (if not two) and pregnancies should be followed up to the time of delivery. The sample size should be very large (an order of magnitude of thousands women) to allow the inclusion of a consistent group of women with fibroids and the possibility to perform reliable subgroup analyses based on location and size of the lesions. Overall, this is a very complex and expensive study and may be considered only in the context of a broader study on the determinants of natural fertility (assessing also the effects of endometriosis, semen quality, diet…).

CONCLUSIONS

The impact of fibroids on natural fertility remains controversial. Epidemiological studies are inconclusive. Given the high prevalence of these lesions in women seeking pregnancy in Western society, robust evidence is pressingly needed. Moreover, new studies should handle the problem of heterogeneity of fibroids and provide information according to number, dimension and location.

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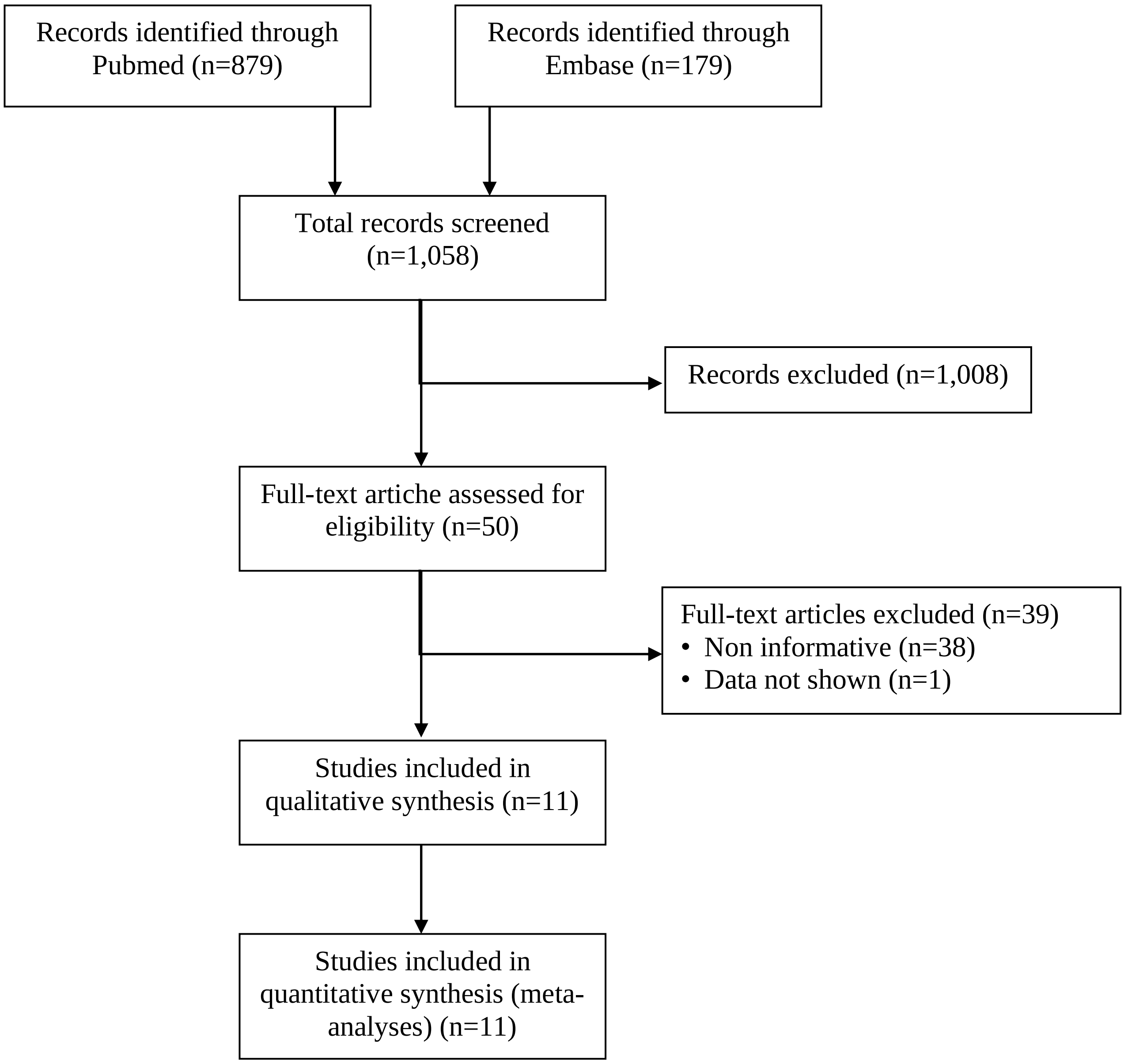
Figure legends

Figure 1: Flow-chart of the process of selection of the studies.

Figure 2: Meta-analyses investigating the relation between fibroids and infertility. The first (*upper panel, Figure A*) grouped the three cohort studies comparing the frequency of a history of infertility in women with and without fibroids (Marshall *et al*., 1998; Wise *et al*., 2004; Templeman *et al*., 2009). The common Incidence Rate Ratio (IRR) of developing fibroids in women with a history of infertility was 1.20 (95%CI: 0.98-1.47). The second meta-analysis (*middle panel, Figure B*) grouped the six case-control or cross-sectional studies investigated the association between a history of infertility and fibroids (Parazzini *et al*., 1996; Bulletti *et al*., 1999; Faerstein *et al*., 2001; Wellons *et al*., 2008; Yasui *et al*., 2018; Egbe *et al*., 2020). The common odds ratio (OR) of carrying fibroids in women with a history of infertility was 2.18 (95%CI: 1.47-3.25). Finally, the third meta-analysis (*lower panel, Figure C*) grouped the two studies comparing the presence of fibroids in fertile and subfertile pregnant women (Johnson *et al*., 2012; Karlsen *et al*., 2019). The common Odds Ratio (OR) of subfertility in women carrying fibroids was 1.93 (95%CI: 0.89-4.18).

Figure 3: Meta-analysis of the four studies whose study design foresaw the recruitment of women with the concomitant presence of fibroids and infertility (Bulletti et al., 1999; Johnson *et al*., 2012; Karlsen *et al*., 2020; Egbe *et al*., 2020). The common OR of fibroids in subfertile women was 3.54 (95%CI: 1.55-8.11).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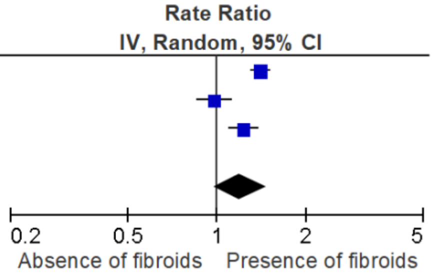
Records excluded (n=1,008)
Full-text artiche assessed for eligibility (n=50)
Full-text articles excluded (n=39)
• Non informative (n=38)
• Data not shown (n=1)
Studies included in qualitative synthesis (n=11)
Studies included in quantitative synthesis (meta-analyses) (n=11)

A

Study	log[Rate Ratio]	SE	Weight	Rate Ratio IV, Random, 95% CI
Marshall 1998	0.34	0.04	35.2%	1.40 [1.30, 1.52]
Wise 2004	-0.02	0.07	31.7%	0.98 [0.85, 1.12]
Templeman 2009	0.21	0.06	33.0%	1.23 [1.10, 1.39]
Total (95% CI)			**100.0%**	**1.20 [0.98, 1.47]**

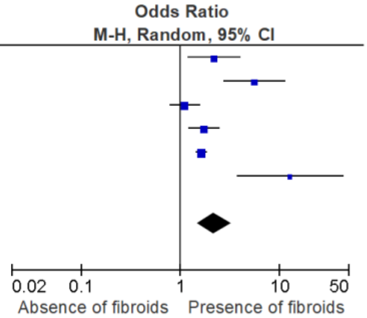
Heterogeneity: Tau² = 0.03; Chi² = 20.29, df = 2 (P < 0.0001); I² = 90%
Test for overall effect: Z = 1.78 (P = 0.07)



B

Study	Presence of fibroids Events	Total	Absence of fibroids Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Parazzini 1996	24	621	19	1051	15.4%	2.18 [1.19, 4.02]
Bulletti 1999	94	106	62	106	13.6%	5.56 [2.72, 11.36]
Faerstein 2001	74	318	85	394	20.3%	1.10 [0.77, 1.57]
Wellons 2008	111	190	134	299	20.0%	1.73 [1.20, 2.50]
Yasui 2018	323	1594	1655	12259	23.5%	1.63 [1.43, 1.86]
Egbe 2020	20	23	79	230	7.2%	12.74 [3.67, 44.19]
Total (95% CI)		**2852**		**14339**	**100.0%**	**2.18 [1.47, 3.25]**
Total events	646		2034			

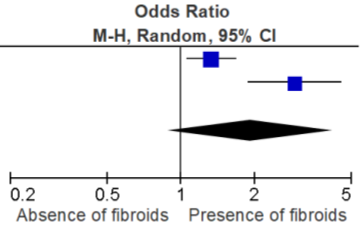
Heterogeneity: Tau² = 0.17; Chi² = 27.44, df = 5 (P < 0.0001); I² = 82%
Test for overall effect: Z = 3.84 (P = 0.0001)



C

Study	Presence of fibroids Events	Total	Absence of fibroids Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Johnson 2012	131	324	903	2676	53.0%	1.33 [1.05, 1.69]
Karlsen 2019	28	92	11357	87358	47.0%	2.93 [1.88, 4.57]
Total (95% CI)		**416**		**90034**	**100.0%**	**1.93 [0.89, 4.18]**
Total events	159		12260			

Heterogeneity: Tau² = 0.28; Chi² = 9.49, df = 1 (P = 0.002); I² = 89%
Test for overall effect: Z = 1.67 (P = 0.10)



Study	Presence of fibroids Events	Presence of fibroids Total	Absence of fibroids Events	Absence of fibroids Total	Weight	Odds Ratio M-H, Random, 95% CI
Bulletti 1999	94	106	62	106	24.7%	5.56 [2.72, 11.36]
Johnson 2012	131	324	903	2676	29.5%	1.33 [1.05, 1.69]
Karlsen 2019	28	92	11357	87358	27.8%	2.93 [1.88, 4.57]
Egbe 2020	20	23	79	230	18.0%	12.74 [3.67, 44.19]
Total (95% CI)		**545**		**90370**	**100.0%**	**3.54 [1.55, 8.11]**
Total events	273		12401			

Heterogeneity: Tau² = 0.59; Chi² = 29.93, df = 3 (P < 0.00001); I² = 90%
Test for overall effect: Z = 2.99 (P = 0.003)

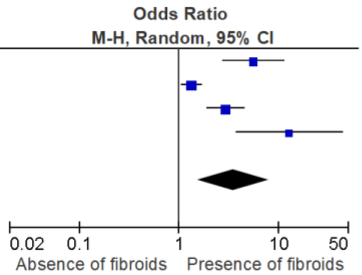


Table 1. Main sources of confounders in studies investigating the relation between fibroids and infertility.

Issue	Citations
Parity displays a strong protective effect on fibroids development.	Wise and Laughlin-Tommaso, 2016; Stewart *et al*., 2018.
Fibroids increase with woman age.	Wise and Laughlin-Tommaso, 2016; Stewart *et al*., 2018.
Infertility increases with woman age.	Schmidt *et al*., 2012; ESHRE Capri Workshop Group, 2017.
Fibroids are associated with clinical conditions causing infertility such as endometriosis.	Hemmings *et al*., 2004; Uimari *et al*., 2011; Capezzuoli *et al*., 2020.
Fibroids are heterogeneous and different type of lesions can affect fertillity differently.	Munro *et al*., 2011; Stewart *et al*., 2016.
A minority of fibroids cause pain symptoms and cycle disturbances. Most fibroids cannot be detected without the use of imaging techniques.	Lumsden *et al*., 2015; Stewart *et al*., 2016.
Natural history of the fibroids is long-lasting and unpredictable.	Mavrelos *et al*., 2011; Armbrust *et al*., 2018.

Table 2. Characteristics of the included studies

Authors, year	Country	Period	Recruitment	Study design	Fibroid assessment	Submucosal lesions	Causes of infertility	Outcome	Details	Quality of evidence [a]
Parazzini *et al*., 1996	Italy	1986-93	Prospective	Case-control	Surgery	Included	Not evaluated	History of infertility	Cases were women operated for fibroids (n=597), controls were women admitted in the hospital for non-gynecological conditions (n=1,032). Subgroup analyses for those aged < 45 years was done.	7
Marshall *et al*., 1998	United States	1989-93	Prospective	Cohort	Self-reported	Included	Evaluated but not analyzed	History of infertility	Nurses' Health study II cohort. Selection of women without fibroids at baseline. Comparison between those who did (n=3,006) and did not (n=92,055) develop fibroids during the following 4 years.	6
Bulletti *et al*., 1994	Italy	1991-96	Prospective	Cross-sectional	Surgery and US	Included	Evaluated and if present case excluded	Natural conception	Infertile women with unremarkable infertility work-up who underwent laparoscopy. Comparison of the subsequent chances of pregnancy in the next 6 months between women with (n=106) and without (n=106) fibroids.	n.a.
Faerstain *et al*., 2001	United States	1990-94	Prospective	Nested case-control	Surgery or US	Included	Not evaluated	History of infertility	Baltimore Women's Health study. Selection of women without fibroids at baseline. Selection of incident cases (n=318) over a 3.5 years period. Controls (n=394) were matched by age and study period. Data collected by phone.	9
Wise *et al*., 2004	United States	1997-2001	Prospective	Cohort	Surgery or US	Included	Evaluated but not analyzed	History of infertility	Black Women's Health Study. Selection of women without fibroids at baseline. Comparison between those who who did (n=2,279) and did not (n=20,616) develop fibroids during the following 4 years.	6
Wellons *et al*., 2008	United States	1985-2002	Prospective	Cross-sectional	US	Included	Evaluated but not analyzed	Presence of fibroids	CARDIA Women's Study. Follow-up at 16 years after inclusion. Comparison of ever infertile (n=190) and never infertile (n=299) women.	n.a.
Templeman *et al*., 2009	United States	1995-2006	Prospective	Cohort	Surgery	Included	Only fertility drugs use	History of infertility	California Teatchers Study. Cohort recruited in 1995-96. Identification of incident cases of fibroids (n=1,790) with hospital discharge records. Controls were non operated women (n=78,414)	8
Johnson *et al*., 2012	United States	n.r.	Prospective	Cross-sectional	US	Included	Not evaluated	Presence of fibroids	Right from the Start study. Recruitment of pregnant women before 13 weeks' gestation. Comparison between women who reported a duration of pregnancy seeling ≤ 3 months (n=1,966) or > 3 months (n=1,034). ART pregnancies excluded.	n.a.
Yasui *et al*., 2018	Japan	2001-07	Prospective	Cross-sectional	Self-reported	Included	Not evaluated	History of infertility	Japan Nurses' Health Study. Women who did (n=1,690) and did not (n=12,99) report fibroids were compared. Cases were confirmed based on a follow-up questionnaire mailed in 2012.	n.a.

Karlsen *et al*., 2020	Denmark	1996-2002	Retrospective	Cohort	Diagnostic or surgical codes	Included	Not evaluated	Presence of fibroids	Linking registers of medical diagnoses and pregnancy. Comparisons of time to pregnancy (> or < 12 months) between women who were diagnosed fibroids before, during or within one year after pregnancy (n=124) and those who were not (91,292). ART pregnancies included.	8
Egbe *et al*., 2020	Cameroon	2016-2017	Prospective	Case-control	Not reported	Included	Evaluated and if present case excluded	Presence of fibroids	Women with bilateral tubal occlusion based on hysterosalpingography were recruited as cases (n=77). Controls were pregnant women refering for antenatal care (n=154).	4

PCOS: Polycistic Ovary Sindrome. US: United States. n.r.: not reported. US: Ultrasound. ART: Assisted Reproductive Technique.

[a] Based on the Newcastle-Ottawa Scale for Assessing the Quality of non-randomized studies

Table 3. Main results of the selected studies

Study	Outcome	Main result	Additional Subgroup analyses
Parazzini *et al*., 1996	Adj. RR of a history of infertility in women carrying fibroids	2.0 (1.1-3.7)	In women ≤ 45 years, Adj. RR was 2.0 (1.2-5.6)
Marshall *et al*., 1998	Adj. RR of a history of infertility in women developping fibroids	1.28 (1.17-1.41)	In nulliparous women, Adj. RR was 1.35 (1.14-1.59)
Bulletti *et al*., 1999	Crude OR. No adjustments made.	2.2 (1.1-4.7)	In women with ≥3 fibroids (n=86), the OR was 2.6 (1.2-1.7).
Faerstain *et al*., 2001	Adj. OR of a history of infertility in women carrying fibroids	1.2 (0.8-1.8)	In women ≤ 25 years with submucosal lesions, Adj. OR was 7.2 (2.0-26.0)
Wise *et al*., 2004	Adj. IRR of a history of infertility in women carrying fibroids	0.9 (0.8-.1.1)	In women ≤ 25 years, Adj. IRR was 1.2 (0.9-1.4)
Wellons *et al*., 2008	Adj. OR of fibroids in ever infertile women	1.54 (1.02-2.31)	Similar trends when focussing separately on black or white women. Adj ORs were 1.69 (0.92-3.10) and 1.48 (0.82-2.66), respectively.
Templeman *et al*., 2009	Adj. RR of a history of infertility in women carrying fibroids	1.14 (1.02-1.28)	Using multivariate model for best predictors, the Adj. RR became 1.23 (1.10-1.38)
Johnson *et al*., 2012	Adj. OR of fibroids in subfertile women	1.0 (0.8-1.1)	For submucosal lesions, the Adj. OR was 1.1 (0.8-1.4). For multiple lesions, it was 1.0 (0.7-1.3). For largest lesions, it was 0.9 (0.7-1.3).
Yasui *et al*., 2018	Adj. OR of a history of infertility in women carrying fibroids	1.64 (1.38-1.95)	None. The study was designed to assess the correlation with hypertension and diabetes.
Karlsen *et al*., 2020	Adj. OR of fibroids in subfertile women	1.67 (1.05-6.68)	Excluding ART pregnancies, the Adj. OR was 1.34 (0.70-2.61)

Egbe *et al*., 2020	Adj. OR of fibroids in women with tubal infertility	62.35 (4.84-803.18)	None.

RR: Relative Risk, OR: Odds Ratio, IRR: Incidence rate ratio. Adj.: Adjusted. Data is reported as estimate (OR, IRR, RR) and (95%CI)