The impact of endometriosis and adenomyosis on the female reproductive system

The impact of endometriosis and adenomyosis on the female reproductive system: risks and management approaches

Konstantinos Nirgianakis

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Thesis

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Contents

Abbreviations:

ART	(Assisted Reproductive Technology)
BMI	(Body Mass Index)
DIE	(Deep Infitrating Endometriosis)
ER	(Estrogen Receptor)
IL-6	(Interleukin-6)
ICSI	(Intra-Cytoplasmatic Sperm Injection)
IUGR	(Intrauterine Growth Restriction)
IVF	(In Vitro Fetrilization)
GnRHa	(Gonadotropin-Releasing Hormone analogues)
GnRHR	(GnRH Receptor)
MD	(Mean Differences)
MRI	(Magnetic Resonance Imaging)
OMA	(Ovarian Endometioma)
OPG	(Osteoprotegerin)
OR	(Odds Ratio)
PA	(Placenta Abruptio)
PF	(Peritoneal Fluid)
PP14	(Glycodelin)
PPH	(Postpartum Hemorrhage)
PP	(Placenta Previa)
RANTES	(Regulated on Activation Normal T cell Expressed and Secreted)
RR	(Risk Ratio)
rASRM	(revised American Society for Reproductive Medicine)
SD	(Standard Deviation)
SGA	(Small for Gestational Age)
SUP	(Superficial Peritoneal Endometriosis)
TNF-a	(Tumor Necrosis Factor-a)
TVS	(Transvaginal Ultrasound)

Chapter 1
General introduction and aims

Endometriosis- a highly prevalent chronic disorder with a serious health impact- therapeutic challenges

Endometriosis is characterized by the growth of endometrial-like tissue outside the uterine cavity. It is a highly prevalent chronic estrogen-dependent disorder observed in women of reproductive age [1-3]. There is a considerable heterogeneity both in phenotype and clinical outcomes that vary from no symptoms to severe pain and/or subfertility often leading to a significant reduction in quality of life [4]. Moreover, the economic impact is substantial, as chronic and debilitating pain from endometriosis may hinder work productivity, while infertility can cause major psychosocial, emotional and financial strain to affected women and their partners [5]. A large multicenter study across Europe, UK and the USA found that the total cost per woman with endometriosis per year was €9.579. The bulk of costs (€6.298) was due to the absence from work, with the economic burden of endometriosis being similar to or even higher than other chronic disease burdens such as heart disease and diabetes mellitus [5]. As a result, national action plans have been declared with the aim to improve the quality of life for individuals living with endometriosis, including a reduction in the impact and burden of disease at individual and population levels [6].

Current treatment options include mainly hormonal based therapies and laparoscopic surgical excision of the endometriotic lesions. Laparoscopic surgery is indeed associated with decreased overall pain, both at 6 and 12 months after surgery [7]. However, despite complete removal of endometriotic tissue, a high proportion of patients will require additional surgery due to endometriosis recurrence. In a recent UK population-based report, 48% of patients with endometriosis received surgical treatment. Approximately one-fifth of these patients required further surgical treatment within 3 years of the index procedure [8]. Other studies have reported total recurrence rates of 21.5% and 40-50% at 2 and 5 years, respectively [9, 10].

Due to the high rate of recurrence after surgery, a new approach on the management of endometriosis has been proposed lately with the aim to avoid repeated surgeries [11] (Figure 1). According to current guidelines, endometriosis-related pain should be empirically treated with adequate analgesia and combined oral contraceptives or progestins prior to definitive laparoscopic diagnosis [12]. Adjuvant hormonal therapy is also advised after laparoscopic surgery to avoid disease recurrence [13, 14]. Unfortunately, adjuvant hormonal therapy is often accompanied by significant, unwanted side effects and treatment failure in about 30% of the patients resulting in a non-adequate reduction in endometriosis-associated pain [15]. In another recent study, 45.4% of the patients have been reported to be unsatisfied with their medical treatment [16] while high treatment discontinuation rates have been observed [17].

Therefore, it is apparent that the major goal of the long-term endometriosis management should be the avoidance of surgical recurrences and the selection of the appropriate long-lasting medical treatment based on its effectiveness and tolerability. The identification of specific risk factors for disease recurrence after surgery as well as medical treatment ineffectiveness and intolerance would be mandatory for an individualized treatment approch. Moreover, considering that current therapeutic options are non-curative and may not align with women's

reproductive goals, improved medical or complementary treatments of endometriosis and associated symptoms are urgenty needed.

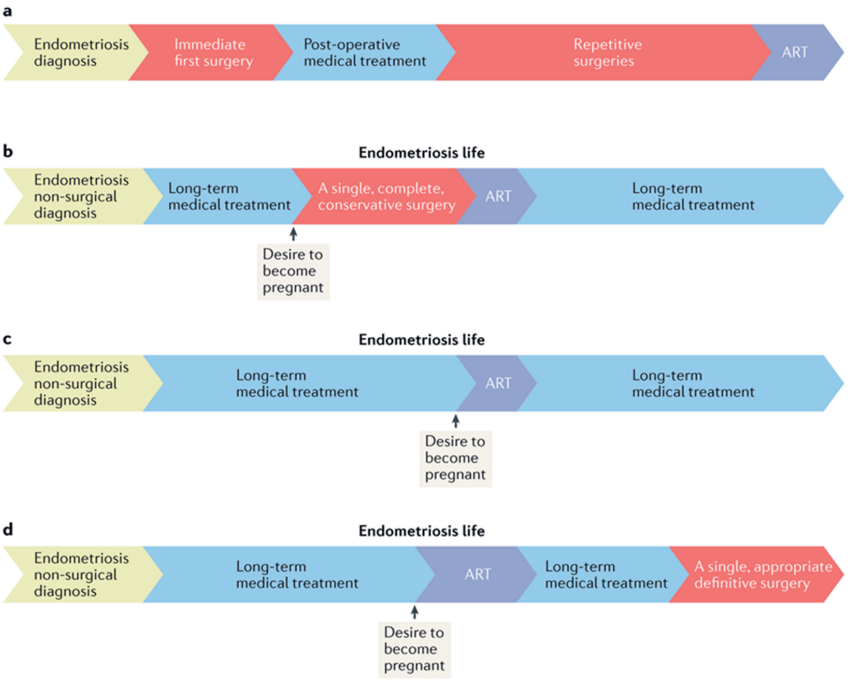

Figure 1. Previous and modern approaches for management of endometriosis (adopted from Chapron et al[11])

a. The conventional and current approach followed by most clinical centres for endometriosis management. b. Suggestion for a modern approach that takes into account endometriosis as a lifelong condition (endometriosis life). This is the first option that can be followed if the patient wishes to become pregnant but is unable to do so spontaneously. c. Option that can be followed if a patient refuses or is unsuitable for surgery. In this context, the patient can be given ART without previous endometriosis surgery. d. For this option, surgery can be provided at the end of the treatment process for patients with pain for whom hormonal treatment is ineffective and/or poorly tolerated and for those who no longer wish to undergo medical treatment.

Endometriosis - a chronic inflammatory disorder and the impact of current therapeutics on inflammation

There are different theories on how endometriosis occurs while the most widely accepted one is the theory of retrograde menstruation [18]. Once endometriosis lesions are established in the peritoneal cavity, they may grow under estrogen influence and secrete various chemokines attracting immune cells and creating an inflammatory microenvironment [19] that facilitates the pathogenesis of endometriosis and may explain pain and subfertility symptoms [20]. Hence, considerable scientific effort has been made over the last decades at examining inflammatory biomarkers in patients with endometriosis [21]. Elevated concentrations of numerous molecules have been reported in the peritoneal fluid of women with

endometriosis [22-26] and investigated as possible non-invasive biomarkers for diagnosis [27-29] or potential targets for the treatment of endometriosis [30-33].

Current treatments for endometriosis such as progestins and gonadotropin releasing hormone analogs (GnRHa) target the estrogen dependence of the disease. Both GnRHa and progestins have been shown to significantly reduce endometriosis-related pain [34-38]. They create a hypo-estrogenic and hyper-progestogenic environment via the inhibition of ovarian follicle development and the subsequent reduction in estrogen production and serum concentrations. However, in addition to estrogen dependence, the inflammatory microenvironment of endometriotic lesions can significantly contribute to both disease progression [39] and symptomatology [20], and therefore, may also represent a viable target for the treatment of endometriosis that has not yet been fully explored [40]. Previous evidence suggests GnRHa modulate the peritoneal microenvironment by decreasing the concentrations of some angiogenic and growth factors, as well as cytokines [41]. Moreover, GnRHa may be able to work directly on extra-pituitary tissue such as the endometrium and the ovary [42-44].

Types of endometriosis- adenomyosis

Endometriotic lesions are separated into three distinct categories: superficial peritoneal lesions (SUP), ovarian endomertriomas (OMA) and deep infiltrating endometriosis (DIE), all of which can exist independently or simultaneously. The least severe form of the disease is SUP, in which superficial endometrial lesions occur on the peritoneum (Figure 2A). OMA present as ovarian cysts usually with concomitant adnexal adhesions that may affect fertility [45] (Figure 2B). DIE lesions are characterized by penetration in excess of 5 mm under the peritoneal surface [46]. They are found in many locations, most commonly in the rectouterine pouch [47], and can involve uterosacral ligaments, the posterior vaginal wall, the anterior rectal wall, and in most severe cases, extend laterally with ureteral involvement [48] (Figure 2C). In addition, endometriosis can occur in extragenital locations, for example, pleural, diaphragmatic or umbilical. Although patients with SUP may suffer from pelvic pain, OMA and DIE generally cause heavier symptoms, have more serious long-term complications and are more difficult to manage [49, 50].

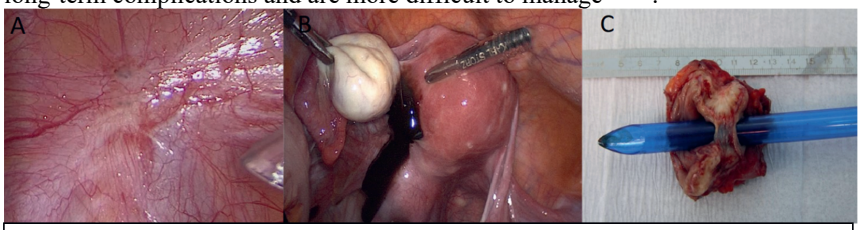

Figure 2. Types of endometriosis. Appearance at laparoscopy.
A. Superficial peritoneal endometriosis (SUP). B. Ovarian endometriosis (OMA). C. Deep infiltrating endometriosis (DIE) in the rectum.

Adenomyosis, characterized by the presence of endometrial tissue within the myometrium, is an enigmatic gynecological disorder with an estimated prevalence of 20-35% in histological series post hysterectomy [51, 52]. It is also a very heterogeneous disease both in anatomical and clinical phenotype varying from normally sized to much enlarged uterus [53] (Figure 3) and from heavy dysmenorrhea

and hypermenorrhea to no symptoms [54] while it is frequently coexistent with endometriosis [55]. Although the pathogenesis of both endometriosis and adenomyosis is not well established, both are the consequence of ectopic localization of endometrial cells. Importantly, adenomyosis contributes, independently of endometriosis, to pain [55], infertility [56] and bleeding (including menorrhagia and metrorrhagia) [57], and has substantial negative effects on the quality of life [58].

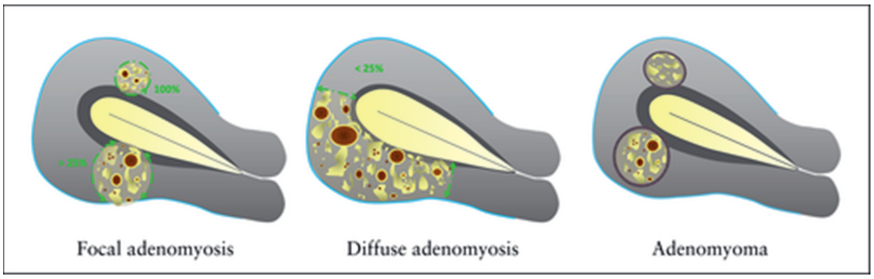

Figure 3. Types of adenomyosis (adopted from Van den Bosch et al [53])

The impact of endometriosis and adenomyosis on pregnancy and delivery

The negative impact of endometriosis on fertility is well known while the involved pathophysiologic mechanisms have been previously described [59] (Figure 4). However, once a patient gets pregnant the endometriosis symptoms appear to improve. This has led to the common myth that having a child will 'fix' endometriosis. The fact is that this improvement is often only temporary due to changes in hormone levels and symptom recurrence after the end of pregnancy can occur.

On the other hand, a negative impact of endometriosis on pregnancy and delivery outcomes has increasingly being recognized. More precisely, a series of controlled observational studies have shown increased pregnancy and delivery complications in patients with endometriosis [60-67] that was confirmed in a systematic meta-analysis [68].

However, most of these studies neither focus on specific endometriosis subtypes, nor do they provide information on surgical treatment and subsequent reproductive performance. This is crucial as pregnancy complications may differ based on the endometriotic subtype [69], or mode of surgery. Moreover, these studies have mainly examined pregnancy but not critical delivery outcomes, such as the rate of failed vaginal delivery or severe birth trauma. As a result, the proper delivery management of these patients remains unclear. One recent study found increased risk of obstetrical complications (preterm birth, placenta previa, hypertension, cesarean delivery complications) in women with untreated posterior DIE [70]. It has not yet been examined if a similar risk persists after complete excision of DIE [71], which is regularly performed in specialized centers with many women achieving pregnancy after surgery [72].

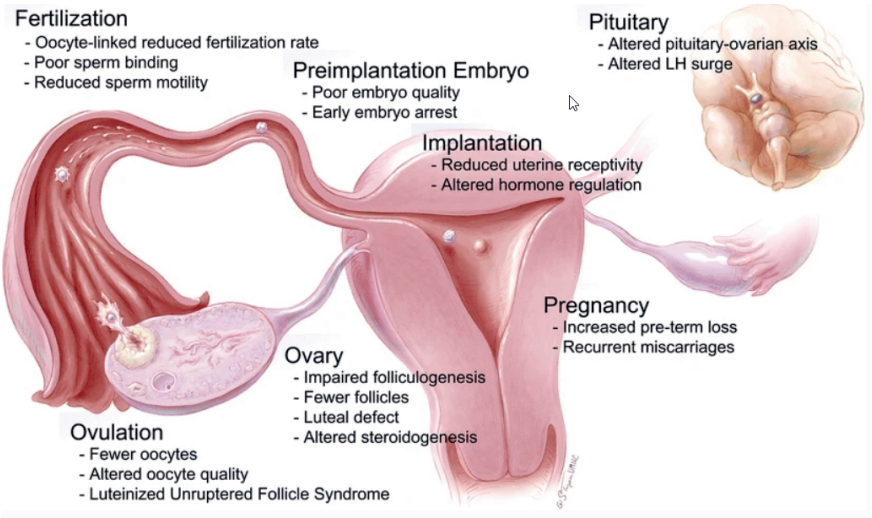

Figure 4. Factors associated with reduced fertility in women with endometriosis (adopted from Stilley et al[59])

Adenomyosis was also reported to be similarly associated with adverse fertility, pregnancy and neonatal outcomes in a recent systematic review [73]. However, this review did not include all eligible studies and no sensitivity analysis according to possible confounders such as the age, number of previous pregnancies, previous mode of delivery and co-existence of endometriosis was performed. Most importantly, pregnancy outcomes were not evaluated based on the method of conception, which represents another possible bias considering that ART is an independent risk factor for pregnancy complications [74] and that many pregnancies in patients with adenomyosis result only after ART.

It is of major importance to identify potential pregnancy and delivery complications and establish evidence-based management policies in these specific groups of patients.

Research questions and hypotheses

Over the past few years, specific challenges in the management of endometriosis and adenomyosis such as disease recurrence and hormonal treatment resistance as well as implications on pregnancy and delivery have been increasingly recognized [14, 68, 75].

However, since endometriosis is a highly heterogenic disease, recurrence might be more associated with certain endometriosis lesions than others. Moreover, the identification of risk or prognostic factors for disease recurrence could lead to a better understanding of the involved mechanisms or to a management modification with the aim to reduce the risk. Finally, it is unknown if the negative impact of endometriosis on the pregnancy outcomes could be ameliorated though the previous endometriosis surgery or specific medical treatments and how adenomyosis, which is very often

coexistent in patients with endometriosis, affects the pregnancy and delivery. These concerns formed the basis for the research hypothesis. We therefore aimed to address the below research questions:

1. Is there an evolution of endometriosis lesion subtypes over each recurrence and do certain lesions have different recurrence potential?

In chapter 2, we characterized the endometriosis lesion subtypes in first and recurrent surgeries, examined their evolution over each recurrence and compared the time required for subsequent surgery based on the initial lesion subtype. This was tested in large cohort of patients who underwent surgery for endometriosis in the Department of Gynecology and Obstetrics, University of Bern.

2. Which patient characteristics are associated with recurrence after surgery for endometriosis?

In chapter 3, we examined potential risk factors for recurrence after surgery in patients with bowel endometriosis. For that kind of severe endometriosis, patients will often need a segmental bowel resection so that all deep infiltrating endometriosis lesions can be excised. Given the risks associated with such complex surgeries, it is very important to identify which patients are at risk for recurrence. To perform that we evaluated several clinical and histological parameters as possible risk factors for disease recurrence in a cohort of patients who underwent laparoscopic segmental bowel resection at the Endometriosis clinic, University of Bern.

3. Given the inflammatory nature of endometriosis, which is the effect of GnRHa on the inflammatory microenvironment of the peritoneal cavity?

In chapter 4, we assessed what effect GnRHa have on the endometriosis-associated inflammation given that a number of inflammatory markers have been previously detected elevated in the peritoneal fluid of patients with endometriosis. We therefore analyzed the concentration of several molecules in the peritoneal fluid of women with endometriosis, and compared these concentrations between women with and without GnRHa treatment prior to surgery. This provides deeper understanding of the mechanisms involved in GnRHa actions and suggests a potential GnRHa role to reduce endometriosis and adenomyosis –associated pregnancy complications.

4. Which is the independent effect of endometriosis in patients undergoing assisted reproductive technology (ART) on the pregnancy outcome?

In chapter 5, we studied whether endometriosis independently correlates with placental complications in patients undergoing ART. This is crucial since ART is also associated with a higher risk of adverse pregnancy outcomes [74, 76] and endometriosis could consist an additional risk factor. To shed light on this issue we performed a systematic review and meta-analysis to compare the incidence of placental disorders in women with and without endometriosis achieving pregnancy through ART.

5. Which is the effect of complete surgical excision of the endometriosis on the endometriosis-associated negative pregnancy and delivery outcome?

In chapter 6, we studied the pregnancy and delivery outcomes in women with previously excised posterior deep infiltrating endometriosis (DIE). We wished to examine a) if surgical excision of DIE prior to pregnancy could counteract the endometriosis-associated pregnancy risks and b) if patients with previous excision of DIE are at a high risk of vaginal delivery complications. We examined that by performing a 1:3 case-control study while the case and control groups were matched for age, parity, previous cesarean section, and mode of conception.

6. Which is the association of adenomyosis with or without coexistent endometriosis with the pregnancy, delivery and neonatal outcome?

In chapter 7, we aimed to a) investigate the association of adenomyosis with fertility outcomes based on the stimulation protocol for the ART and adjusted to possible confounders, b) assess the association of adenomyosis with pregnancy and neonatal outcomes separately after natural and ART conception as well as adjusted to other possible confounders and c) determine if certain adenomyosis subtypes have a greater impact than others on the reproductive course. Therefore, we conducted a systematic review and meta-analysis including all observational studies comparing the reproductive course of patients with and without adenomyosis.

References

1. Janssen EB, Rijkers AC, Hoppenbrouwers K, Meuleman C, D'Hooghe TM. Prevalence of endometriosis diagnosed by laparoscopy in adolescents with dysmenorrhea or chronic pelvic pain: a systematic review. *Human reproduction update* 2013, 19: 570-82.

2. Gylfason JT, Kristjansson KA, Sverrisdottir G, Jonsdottir K, Rafnsson V, Geirsson RT. Pelvic endometriosis diagnosed in an entire nation over 20 years. *American journal of epidemiology* 2010, 172: 237-43.

3. Bougie O, Yap MI, Sikora L, Flaxman T, Singh S. Influence of race/ethnicity on prevalence and presentation of endometriosis: a systematic review and meta-analysis. *BJOG : an international journal of obstetrics and gynaecology* 2019.

4. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. *Human reproduction* 2007, 22: 266-71.

5. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I *et al.* The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Human reproduction* 2012, 27: 1292-9.

6. Australia Co. National Action Plan for Endometriosis. In: Health Do, ed., 2018.

7. Duffy JM, Arambage K, Correa FJ, Olive D, Farquhar C, Garry R *et al.* Laparoscopic surgery for endometriosis. *The Cochrane database of systematic reviews* 2014: Cd011031.

8. Cea Soriano L, Lopez-Garcia E, Schulze-Rath R, Garcia Rodriguez LA. Incidence, treatment and recurrence of endometriosis in a UK-based population analysis using data from The Health Improvement Network and the Hospital Episode Statistics database. *The European journal of contraception & reproductive health care : the official journal of the European Society of Contraception* 2017, 22: 334-43.

9. Guo SW. Recurrence of endometriosis and its control. *Human reproduction update* 2009, 15: 441-61.

10. Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Vigano P, Fedele L. The effect of surgery for symptomatic endometriosis: the other side of the story. *Human reproduction update* 2009, 15: 177-88.

11. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nature Reviews Endocrinology* 2019, 15: 666-82.

12. . Practice bulletin no. 114: management of endometriosis. *Obstet Gynecol* 2010; 116:223-36.

13. Lee SR, Yi KW, Song JY, Seo SK, Lee DY, Cho S *et al.* Efficacy and Safety of Long-Term Use of Dienogest in Women With Ovarian Endometrioma. *Reproductive sciences* 2018, 25: 341-6.

14. Somigliana E, Vercellini P, Vigano P, Benaglia L, Busnelli A, Fedele L. Postoperative medical therapy after surgical treatment of endometriosis: from adjuvant therapy to tertiary prevention. *Journal of minimally invasive gynecology* 2014, 21: 328-34.

15. Becker CM, Gattrell WT, Gude K, Singh SS. Reevaluating response and failure of medical treatment of endometriosis: a systematic review. *Fertility and sterility* 2017, 108: 125-36.

16. Lukas I, Kohl-Schwartz A, Geraedts K, Rauchfuss M, Wölfler MM, Häberlin F *et al.* Satisfaction with medical support in women with endometriosis. *PloS one* 2018, 13: e0208023.

17. Seracchioli R, Mabrouk M, Frascà C, Manuzzi L, Savelli L, Venturoli S. Long-term oral contraceptive pills and postoperative pain management after laparoscopic excision of ovarian endometrioma: a randomized controlled trial. *Fertility and sterility* 2010, 94: 464-71.

18. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstetrics and Gynecology* 1984, 64: 151-4.

19. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. *Fertility and sterility* 2001, 75: 1-10.

20. McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and nerve fiber interaction in endometriotic pain. *Trends in endocrinology and metabolism: TEM* 2015, 26: 1-10.

21. Zhou WJ, Yang HL, Shao J, Mei J, Chang KK, Zhu R *et al.* Anti-inflammatory cytokines in endometriosis. *Cellular and molecular life sciences : CMLS* 2019, 76: 2111-32.

22. Bersinger NA, von Roten S, Wunder DM, Raio L, Dreher E, Mueller MD. PAPP-A and osteoprotegerin, together with interleukin-8 and RANTES, are elevated in the peritoneal fluid of women with endometriosis. *American journal of obstetrics and gynecology* 2006, 195: 103-8.

23. Ryan IP, Tseng JF, Schriock ED, Khorram O, Landers DV, Taylor RN. Interleukin-8 concentrations are elevated in peritoneal fluid of women with endometriosis. *Fertility and sterility* 1995, 63: 929-32.

24. Hirota Y, Osuga Y, Koga K, Yoshino O, Hirata T, Harada M *et al.* Possible implication of midkine in the development of endometriosis. *Human reproduction* 2005, 20: 1084-9.

25. Nirgianakis K, Grandi G, McKinnon B, Bersinger N, Cagnacci A, Mueller M. Dienogest mediates midkine suppression in endometriosis. *Human reproduction* 2016, 31: 1981-6.

26. Nirgianakis K, McKinnon B, Ma L, Imboden S, Bersinger N, Mueller MD. Peritoneal fluid biomarkers in patients with endometriosis: a cross-sectional study. *Hormone molecular biology and clinical investigation* 2020.

27. Vodolazkaia A, El-Aalamat Y, Popovic D, Mihalyi A, Bossuyt X, Kyama CM *et al.* Evaluation of a panel of 28 biomarkers for the non-invasive diagnosis of endometriosis. *Human reproduction* 2012, 27: 2698-711.

28. Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS *et al.* Blood biomarkers for the non-invasive diagnosis of endometriosis. *The Cochrane database of systematic reviews* 2016: Cd012179.

29. Liu E, Nisenblat V, Farquhar C, Fraser I, Bossuyt PM, Johnson N *et al.* Urinary biomarkers for the non-invasive diagnosis of endometriosis. *The Cochrane database of systematic reviews* 2015: Cd012019.

30. Lv D, Song H, Shi G. Anti-TNF-alpha treatment for pelvic pain associated with endometriosis. *The Cochrane database of systematic reviews* 2010: Cd008088.

31. Ingelmo JM, Quereda F, Acien P. Effect of human interferon-alpha-2b on experimental endometriosis in rats: comparison between short and long series of treatment. *European journal of obstetrics, gynecology, and reproductive biology* 2013, 167: 190-3.

32. Koninckx PR, Craessaerts M, Timmerman D, Cornillie F, Kennedy S. Anti-TNF-alpha treatment for deep endometriosis-associated pain: a randomized placebo-controlled trial. *Human reproduction* 2008, 23: 2017-23.

33. Grandi G, Mueller M, Bersinger N, Papadia A, Nirgianakis K, Cagnacci A *et al.* Progestin suppressed inflammation and cell viability of tumor necrosis factor-alpha-stimulated endometriotic stromal cells. *American journal of reproductive immunology* 2016, 76: 292-8.

34. Brown J, Pan A, Hart RJ. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. *The Cochrane database of systematic reviews* 2010, 2010: Cd008475.

35. Strowitzki T, Marr J, Gerlinger C, Faustmann T, Seitz C. Dienogest is as effective as leuprolide acetate in treating the painful symptoms of endometriosis: a 24-week, randomized, multicentre, open-label trial. *Human reproduction* 2010, 25: 633-41.

36. Strowitzki T, Faustmann T, Gerlinger C, Schumacher U, Ahlers C, Seitz C. Safety and tolerability of dienogest in endometriosis: pooled analysis from the European clinical study program. I*nt J Womens Health* 2015, 7: 393-401.

37. Harada T, Momoeda M, Taketani Y, Aso T, Fukunaga M, Hagino H *et al.* Dienogest is as effective as intranasal buserelin acetate for the relief of pain symptoms associated with endometriosis--a randomized, double-blind, multicenter, controlled trial. *Fertility and sterility* 2009, 91: 675-81.

38. Petraglia F, Hornung D, Seitz C, Faustmann T, Gerlinger C, Luisi S *et al.* Reduced pelvic pain in women with endometriosis: efficacy of long-term dienogest treatment. *Archives of gynecology and obstetric*s 2012, 285: 167-73.

39. Giudice LC, Kao LC. *Endometriosis. Lancet (London, England)* 2004, 364: 1789-99.

40. Hardy DB, Janowski BA, Corey DR, Mendelson CR. Progesterone receptor plays a major antiinflammatory role in human myometrial cells by antagonism of nuclear factor-kappaB activation of cyclooxygenase 2 expression. *Molecular endocrinology (Baltimore, Md)* 2006, 20: 2724-33.

41. Küpker W, Schultze-Mosgau A, Diedrich K. Paracrine changes in the peritoneal environment of women with endometriosis. *Human reproduction update* 1998, 4: 719-23.

42. Borroni R, Di Blasio AM, Gaffuri B, Santorsola R, Busacca M, Viganò P *et al.* Expression of GnRH receptor gene in human ectopic endometrial cells and inhibition of their proliferation by leuprolide acetate. *Molecular and Cellular Endocrinology* 2000, 159: 37-43.

43. Tesone M, Bilotas M, Barañao RI, Meresman G. The Role of GnRH Analogues in Endometriosis-Associated Apoptosis and Angiogenesis. *Gynecologic and obstetric investigation* 2008, 66(suppl 1): 10-8.

44. Kang SK, Choi KC, Cheng KWA, Nathwani PS, Auersperg N, Leung PCK. Role of gonadotropin-releasing hormone as an autocrine growth factor in human ovarian surface epithelium. *Endocrinology* 2000, 141: 72-80.

45. Goodman LR, Goldberg JM, Flyckt RL, Gupta M, Harwalker J, Falcone T. Effect of surgery on ovarian reserve in women with endometriomas, endometriosis and controls. *American journal of obstetrics and gynecology* 2016, 215: 589.e1-.e6.

46. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertility and sterility* 1991, 55: 759-65.

47. Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V *et al.* Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Human reproduction* 2003, 18: 157-61.

48. Knabben L, Imboden S, Fellmann B, Nirgianakis K, Kuhn A, Mueller MD. Urinary tract endometriosis in patients with deep infiltrating endometriosis: prevalence, symptoms, management, and proposal for a new clinical classification. *Fertility and sterility* 2015, 103: 147-52.

49. Vercellini P, Trespidi L, De Giorgi O, Cortesi I, Parazzini F, Crosignani PG. Endometriosis and pelvic pain: relation to disease stage and localization. *Fertility and sterility* 1996, 65: 299-304.

50. Chapron C, Fauconnier A, Dubuisson JB, Barakat H, Vieira M, Breart G. Deep infiltrating endometriosis: relation between severity of dysmenorrhoea and extent of disease. *Human reproduction* 2003, 18: 760-6.

51. Abbott JA. Adenomyosis and Abnormal Uterine Bleeding (AUB-A)-Pathogenesis, diagnosis, and management. *Best practice & research Clinical obstetrics & gynaecology* 2017, 40: 68-81.

52. Vercellini P, Vigano P, Somigliana E, Daguati R, Abbiati A, Fedele L. Adenomyosis: epidemiological factors. *Best practice & research Clinical obstetrics & gynaecology* 2006, 20: 465-77.

53. Van den Bosch T, de Bruijn AM, de Leeuw RA, Dueholm M, Exacoustos C, Valentin L *et al.* Sonographic classification and reporting system for diagnosing adenomyosis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2019, 53: 576-82.

54. Chapron C, Vannuccini S, Santulli P, Abrao MS, Carmona F, Fraser IS *et al.* Diagnosing adenomyosis: an integrated clinical and imaging approach. *Human reproduction update* 2020.

55. Lazzeri L, Di Giovanni A, Exacoustos C, Tosti C, Pinzauti S, Malzoni M *et al.* Preoperative and Postoperative Clinical and Transvaginal Ultrasound Findings of Adenomyosis in Patients With Deep Infiltrating Endometriosis. *Reproductive sciences* 2014, 21: 1027-33.

56. Kunz G, Beil D, Huppert P, Noe M, Kissler S, Leyendecker G. Adenomyosis in endometriosis--prevalence and impact on fertility. Evidence from magnetic resonance imaging. *Human reproduction* 2005, 20: 2309-16.

57. Naftalin J, Hoo W, Pateman K, Mavrelos D, Foo X, Jurkovic D. Is adenomyosis associated with menorrhagia? *Human reproduction* 2014, 29: 473-9.

58. Nelsen LM, Lenderking WR, Pokrzywinski R, Balantac Z, Black L, Pokras S *et al.* Experience of Symptoms and Disease Impact in Patients with Adenomyosis. *The patient* 2018, 11: 319-28.

59. Stilley JAW, Birt JA, Sharpe-Timms KL. Cellular and molecular basis for endometriosis-associated infertility. *Cell and Tissue Research* 2012, 349: 849-62.

60. Takemura Y, Osuga Y, Fujimoto A, Oi N, Tsutsumi R, Koizumi M *et al.* Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. *Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology* 2013, 29: 113-5.

61. Conti N, Cevenini G, Vannuccini S, Orlandini C, Valensise H, Gervasi MT *et al.* Women with endometriosis at first pregnancy have an increased risk of adverse obstetric outcome. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet 2015*, 28: 1795-8.

62. Lin H, Leng JH, Liu JT, Lang JH. Obstetric outcomes in Chinese women with endometriosis: a retrospective cohort study. *Chinese medical journal* 2015, 128: 455-8.

63. Benaglia L, Candotti G, Papaleo E, Pagliardini L, Leonardi M, Reschini M *et al.* Pregnancy outcome in women with endometriosis achieving pregnancy with IVF. *Human reproduction* 2016, 31: 2730-6.

64. Glavind MT, Forman A, Arendt LH, Nielsen K, Henriksen TB. Endometriosis and pregnancy complications: a Danish cohort study. *Fertility and sterility* 2017, 107: 160-6.

65. Mannini L, Sorbi F, Noci I, Ghizzoni V, Perelli F, Di Tommaso M *et al.* New adverse obstetrics outcomes associated with endometriosis: a retrospective cohort study. *Archives of gynecology and obstetrics* 2017, 295: 141-51.

66. Saraswat L, Ayansina DT, Cooper KG, Bhattacharya S, Miligkos D, Horne AW *et al.* Pregnancy outcomes in women with endometriosis: a national record linkage study. *BJOG : an international journal of obstetrics and gynaecology* 2017, 124: 444-52.

67. Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Human reproduction* 2009, 24: 2341-7.

68. Zullo F, Spagnolo E, Saccone G, Acunzo M, Xodo S, Ceccaroni M *et al.* Endometriosis and obstetrics complications: a systematic review and meta-analysis. *Fertility and sterility* 2017, 108: 667-72.e5.

69. Vercellini P, Parazzini F, Pietropaolo G, Cipriani S, Frattaruolo MP, Fedele L. Pregnancy outcome in women with peritoneal, ovarian and rectovaginal endometriosis: a retrospective cohort study. *BJOG : an international journal of obstetrics and gynaecology* 2012, 119: 1538-43.

70. Exacoustos C, Lauriola I, Lazzeri L, De Felice G, Zupi E. Complications during pregnancy and delivery in women with untreated rectovaginal deep infiltrating endometriosis. *Fertility and sterility* 2016, 106: 1129-35.e1.

71. Leone Roberti Maggiore U, Inversetti A, Schimberni M, Viganò P, Giorgione V, Candiani M. Obstetrical complications of endometriosis, particularly deep endometriosis. *Fertility and sterility* 2017, 108: 895-912.

72. Meuleman C, Tomassetti C, D'Hoore A, Van Cleynenbreugel B, Penninckx F, Vergote I *et al.* Surgical treatment of deeply infiltrating endometriosis with colorectal involvement. *Human reproduction update 2011, 17: 311-26.*

73. Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. *Human reproduction update* 2019, 25: 592-632.

74. Qin JB, Sheng XQ, Wu D, Gao SY, You YP, Yang TB *et al.* Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Archives of gynecology and obstetrics* 2017, 295: 285-301.

75. Nirgianakis K, Vaineau C, Agliati L, McKinnon B, Gasparri ML, Mueller MD. Risk factors for non-response and discontinuation of Dienogest in endometriosis patients: A cohort study. *Acta obstetricia et gynecologica Scandinavica* 2020.

76. Karami M, Jenabi E, Fereidooni B. *The association of placenta previa and assisted reproductive techniques: a meta-analysis. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2018, 31: 1940-7.

Chapter 2

Recurrence Patterns after Surgery in Patients with Different Endometriosis Subtypes: A Long-Term Hospital-Based Cohort Study

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Abstract

Recurrence of endometriosis after surgery constitutes a serious challenge. Whether there is an evolution of lesion subtypes with each recurrence and whether certain lesions subtypes tend to recur faster than others is not adequately addressed. Medical records of all patients who underwent surgery for endometriosis between 1997 and 2018 in the Department of Gynecology and Obstetrics, University of Bern, were reviewed. Inclusion criteria was surgically confirmed endometriosis recurrence, defined as a subsequent surgery for endometriosis after a previous complete surgical excision of endometriosis lesions. Three subtypes of endometriosis were defined: superficial peritoneal endometriosis (SUP), ovarian endometrioma (OMA) and deep infiltrating endometriosis (DIE). Time to recurrence and variation in endometriosis subtype between the first and recurrent surgeries were the primary outcome measures. 322 patients with recurrent surgery were identified. For 234 the endometriosis subtype at first surgery was confirmed and classified (SUP = 56, OMA = 124, DIE = 54). No statistically significant difference was found for time to recurrence between lesion subtypes. SUP compared to the other groups had a higher possibility of presenting with SUP at recurrence (OR: 3.65, 95% CI: 1.74-7.51) and OMA compared to the other groups had a higher possibility of presenting with OMA at recurrence (OR: 3.72, 95% CI: 2.04-6.74). Nevertheless, a large number of SUP patients subsequently presented with OMA (10/56: 17.9%) or DIE (27/56: 48.2%) lesions at recurrence. Similarly, a large number of OMA patients subsequently presented with DIE (49/124: 39.5%) lesions at recurrence. In conclusion, although SUP and OMA patients compared to the others are more likely to present with the same subtype at recurrence, increasing lesion subtype severity occurs in a substantial proportion of patients. Time to recurrence is independent from the lesion subtype at first surgery.

Introduction

Endometriosis, characterized by the growth of endometrial-like tissue outside the uterine cavity, is a highly prevalent gynecological disorder of reproductive-aged women worldwide [1-3]. It is a significantly heterogeneous disease, both in phenotype and clinical outcomes that can lead to a significant reduction in quality of life and work productivity [4, 5]. Recommended treatments for endometriosis are either hormonal based therapy or laparoscopic surgical excision depending on response and tolerability to medical treatment, as well as family planning.

Laparoscopic surgery is associated with decreased overall pain, both at 6 and 12 months after surgery [6]. However, despite complete removal of endometriotic tissue a high proportion of patients will require additional surgery due to endometriosis recurrence. In a recent UK population-based report, 48% of patients with endometriosis received surgical treatment. Approximately one-fifth of these patients required further surgical treatment, within 3 years of the index procedure[7]. Other studies have reported total recurrence rates of 21.5% and 40-50% at 2 and 5 years, respectively [8, 9].

Endometriosis lesions are a heterogenous group of lesions that are currently split into three subtypes based on the location and infiltration depth: superficial peritoneal endometriosis (SUP), ovarian endometrioma (OMA) and deep infiltrating endometriosis (DIE) [10, 11]. Although patients with SUP may suffer from pelvic pain, OMA and DIE generally cause heavier symptoms, have more serious long-term complications and are more difficult to manage [12-16], thus considered as more severe endometriosis subtypes. Whether there is an evolution of lesion subtypes over each recurrence and whether certain lesions subtypes recur faster than others is not adequately addressed. Research up to now has been scant, limited to adolescence and with contradictory results [17-21].

The purpose of this study, therefore, was to characterize the lesion subtypes in first and subsequent surgeries, examine their evolution and compare the time required for subsequent surgery based on the initial lesion subtype.

Materials and Methods

The study was prepared according to the "Strengthening the reporting of observational studies in epidemiology" guidelines [22] and was institution review board approved (no. 2017-00952). The electronic medical records were searched for all patients who underwent at least one laparoscopic surgery for endometriosis in the Department of Gynecology and Obstetrics, University of Bern (between January 1997 and October 2018). The initial search for inclusion criteria was performed by one researcher (L.M.) and the medical records identified for inclusion were reviewed by two independent researchers (K.N.) (L.M). Only patients with more than one surgery for endometriosis were included, while surgeries in external hospitals were not excluded. For all surgeries, either visual or histological confirmation of endometriosis was required for inclusion. Unavailable surgical report or undefined surgical technique, incomplete excision of endometriosis lesions and diagnostic surgeries made up the exclusion criteria. Recurrence was defined as subsequent surgery for endometriosis after a previous, complete surgical excision of endometriosis. Recurrence of endometriosis symptoms or recurrence of endometriosis based on clinical suspicion or imaging was not evaluated.

Surgical data, histological results and time to recurrence were collected and analyzed retrospectively. The classification of endometriosis subtype was

performed according to the most severe endometriotic lesion identified [23]. As a result, DIE with concomitant OMA and/or SUP was classified as DIE. OMA with concomitant SUP was classified as OMA.

Surgical Technique

The standardized laparoscopic surgical technique for DIE performed in our clinic has been described previously [24]. SUP was treated by excision via monopolar needle or scissors. OMA was treated by the striping technique.

Statistical Analysis

Median values and range, or mean values and standard deviation (SD) were calculated for continuous variables and percentages for the qualitative variables. The time to recurrence was assessed according to the Kaplan-Meier life-table analysis. A log-rank test was used to compare the recurrence rates between groups. Ordinary one-way ANOVA and Kruskal-Wallis test were used to compare continuous parametric and nonparametric variables, respectively. Fisher's exact test was used to compare the proportion of endometriosis subtypes at each recurrence and to determine whether lesion subtype was more or less severe. Significance was set at a p-value of <0.05. Statistical analysis was carried out with GraphPad Prism version 7.0 (GraphPad Software).

Results

Patient characteristics

Among 1332 patients with surgically diagnosed endometriosis, 322 satisfied both the inclusion and exclusion criteria. For 234 (72.7%), the endometriosis subtype at first surgery was verified from the surgical report. For the remaining 88 patients the endometriosis subtype was unclear. The patient`s characteristics recorded at the initial surgery are summarized in Table 1.

First surgery / Characteristics	SUP (N= 56)	OMA (N= 124)	DIE (N= 54)	Unknown (N=88)	*P*
Age (y ± SD)	27.7 ± 6.4	29.4 ± 5.3	30.1 ± 5.0	29.4 ± 6.6	ns
Median time to second surgery (min-max, months)	30.5 (5-216)	30 (6-244)	36 (4-141)	33.5 (5-190)	ns
First surgery in external hospital	43 (76.8%)	109 (87.9%)	33 (61.1%)	85 (96.6%)	**<0.001***
Second surgery in external hospital	12 (21.4%)	40 (32.3%)	12 (22.2%)	33 (37.5%)	ns
One recurrence	100%	100%	100%	100%	n/a
Two recurrences	18 (32.1%)	52 (41.9%)	18 (33.3%)	44 (50.0%)	ns
Three recurrences	4 (7.1%)	16 (12.9%)	8 (14.8%)	22 (25.0%)	ns
Four recurrences	2 (3.6%)	4 (3.2%)	4 (7.4%)	13 (14.8%)	ns
Five recurrences	0	0	1 (1.9%)	3 (3.4%)	ns

Table 1. Patient's characteristics according to the endometriosis subtype at first surgery

Time to recurrence

The median time to first recurrent surgery, irrespective of lesion subtype, was 32 months (5-244 months) (Fig S1). For patients who underwent a second recurrent surgery, it was performed after an additional 35 months (5-222 months). Surgery for the third and fourth recurrence were performed after 30 (6-160 months) and 34 (5-90 months) months, respectively. The times between surgeries for each recurrence were not statistically significantly different (Fig S2).

For patients categorized in the SUP group, based on their first surgery for endometriosis, the median time to their first recurrence was 30.5 (5-216) months. For patients categorised in the OMA group this time was 30 (6-244) months and for patients categorized in the DIE group this was 36 (4-141) months. The time to recurrence for each lesion subtype was not statistically significantly different (Fig 1).

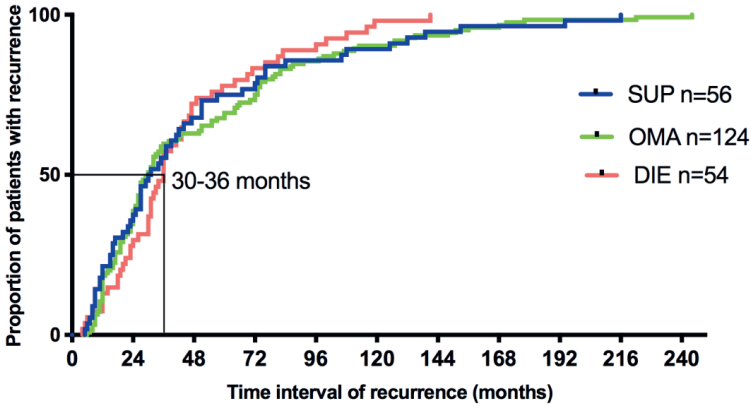

Figure 1. Time to first recurrent surgery according to the initial type of endometriosis. The time to recurrence is illustrated in different colors according to the endometriosis lesion subtype at initial surgery. No statistically significant difference was observed. Abbreviations: SUP, superficial peritoneal endometriosis; OMA, ovarian endometrioma; DIE, deep infiltrating endometriosis.

Recurrent endometriosis subtype, based on subtype at first surgery

Patients that had a SUP at the first surgery were more likely to present again with a SUP at subsequent surgery (17/56: 30.4%), compared to women that originally had an OMA (10/124: 8.1%), or women that originally had a DIE (9/54: 16.7%). This difference was statistically significant (OR: 3.65, 95% CI: 1.74-7.51; p=.001). Similarly, patients that had an OMA at first surgery were more likely, to have an OMA (58/124: 46.8%) at subsequent surgery compared to women that originally had a SUP (10/56: 17.9%), or women that had a DIE (11/54: 20.6%). This difference was statistically significant (OR: 3.72, 95% CI: 2.04-6.74; p<0.0001). Patients that initially presented with DIE showed a trend to also subsequently present with DIE (29/54: 53.7%) at the next surgery. However, compared to the other groups, it was not statistically significantly higher, reflecting the high percentage of patients from the other groups that had DIE at subsequent surgeries (Table 2, Fig 2).

Evolution of endometriosis subtypes over recurrent surgeries

Interestingly, although the above results suggest the lesion subtype present at the first surgery is a good indication of the lesion to expect at the recurrent surgery, the results also show that a substantial proportion of patients with initially SUP or OMA lesions progress to a more severe subtype at recurrence. Of the women initially presenting with SUP 66.1% returned for recurrent surgery with either an OMA (10/56: 17.9%) or DIE (27/56: 48.2%), which when both subtypes were combined as more severe was statistically significant ($p = 0.0295$). Similarly, of the women initially diagnosed with an OMA 39.5% (49/124) returned for recurrent surgery with DIE whereas only 8.1% (10/124) with the less severe SUP (Table 2, Fig 2), which was also statistically significant based on a Fisher exact test of more vs less severe lesions ($p < 0.0001$).

There were two patients (3.6%) in the initially SUP group that had a concomitant hysterectomy at recurrent surgery. One patient (1.8%) underwent hysterectomy during the first surgery, but still required a subsequent surgery for a recurrence of OMA. Six patients (5%) in the initially OMA group underwent a concomitant hysterectomy at recurrent surgery. Three patients (2.4%) underwent a hysterectomy during the first surgery, all three of which had an OMA lesion at subsequent surgery, one of which also had a DIE lesion. Eight patients (15.4%) in the DIE group underwent a concomitant hysterectomy at the second surgery. Two patients (3.7%) underwent hysterectomy during the first surgery and both subsequently presented with DIE at the next surgery; one of which was combined with an OMA. Concomitant hysterectomy at recurrence was significantly more common in the DIE compared to the SUP or OMA group (OR: 3.00, 95% CI: 1.11-7.73; p= .007).

The majority of the OMA identified at subsequent surgeries occurred on the same ovary as the initial surgery (Table 3a). Similarly, the majority of the patients that presented with DIE lesions at first surgery were most likely to have recurrent lesions in the same area at the subsequent surgery (Table 3b).

Of the 322 patients that underwent at least two surgeries for endometriosis 128 (39.8%) had an additional 3rd surgery and 48 (14.9%) a 4th surgery. In these patients, we observed a similar trend with a high proportion of patients presenting with more severe subtypes and in particular DIE lesions at subsequent surgery. The data are presented in the supplemental table 1 (2nd to 3rd surgery) and 2 (3rd to 4th surgery). The discrepancy between the above-referred total numbers of recurrences and the numbers in the supplemental tables is due to some patients for which the lesion subtypes could not be classified, thus not included in the tables. Due to the limited sample numbers a statistical analysis was not performed.

First surgery / Recurrent surgery	**SUP (N= 56) median time to recurrence (min-max)**	**P OR (95% CI)[1]**	**OMA (N= 124) median time to recurrence (min-max)**	**P OR (95% CI)[2]**	**DIE (N= 54) median time to recurrence (min-max)**	**P OR (95% CI)[3]**
SUP	17 (30.4%); 30 (9-194)	**0.001 3.65 (1.74, 7.51)**	10 (8.1%); 28 (7-244)	0.0011 0.28 (0.14, 0.62)	9 (16.7%); 31 (5-116)	ns
OMA	10 (17.9%); 71.5 (6-216)	0.0036	58 (46.8%); 27 (6-222)	**<0.0001**	11 (20.4%); 36 (6-141)	0.021 0.42 (0.2, 0.87)

		0.34 (0.17, 0.73)		**3.72 (2.04, 6.74)**		
DIE	27 (48.2%); 27 (5-139)	ns	49 (39.5%); 51 (8-135)	ns	29 (53.7%); 39 (18-119)	ns
Unknown subtype	2 (3.6%); 31.5 (12,51)	ns	5 (4.0 %); 30 (12-156)	ns	5 (9.3%); 24 (4-31)	ns

Table 2. Evolution of endometriosis from first to recurrent surgery.
In each cell, the number of cases evolving into SUP, OMA, DIE, or unknown lesion subtype at recurrent surgery and their percentage is given. 1: The ORs in this column reflect the possibility of a patient with initially SUP lesions compared to a patient with initially non-SUP lesions (OMA and DIE) to present a certain endometriosis lesion at recurrent surgery. The bold numbers represent the statistically significant higher possibility of SUP patients, compared to non-SUP patients to present with SUP lesions again, in the absence of OMA and DIE at recurrent surgery. 2: The ORs in this column reflect the possibility of a patient with initially OMA lesions compared to a patient with initially non-OMA lesions (SUP and DIE) to present a certain endometriosis lesion at recurrent surgery. The bold marked numbers represent the statistically significant higher possibility of OMA patients, compared to non-OMA patients to present with OMA lesions again in the absence of DIE at recurrent surgery. 3: The ORs in this column reflect the possibility of a patient with initially DIE lesions compared to a patient with initially non-DIE lesions (SUP and OMA) to present SUP, OMA or DIE at recurrent surgery. Abbreviations: SUP, superficial peritoneal endometriosis; OMA, ovarian endometrioma; DIE, deep infiltrating endometriosis; ns, not significant; Min-Max, Minimum-Maximum; OR (95% CI), Odds Ratio (95% Confidence Interval).

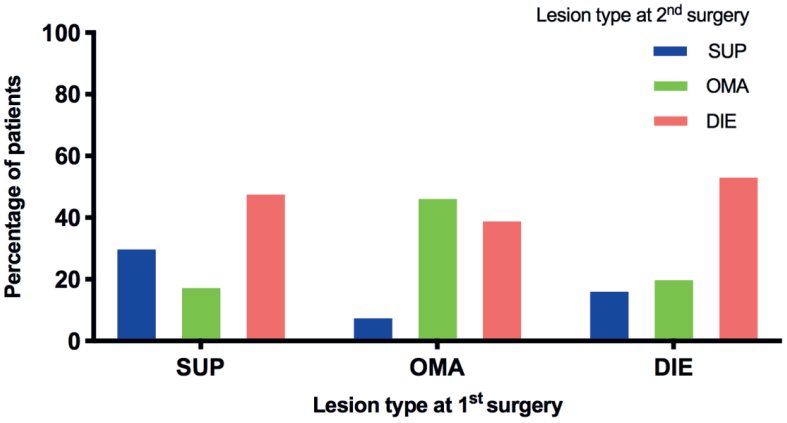

Figure 2. The evolution of SUP, OMA and DIE at first recurrence. Legend: Graphical illustration of Table 2. The evolution of the lesions from the first to recurrent surgery is presented. Each group of patients is split into 3 colored columns with each color representing a certain lesion subtype at recurrent surgery. Abbreviations: SUP, superficial peritoneal endometriosis; OMA, ovarian endometrioma; DIE, deep

Location of OMA at 1st surgery / Location of OMA at 2nd surgery	Bilateral n=13 (22.4%)	Unilateral left n=21 (36.2%)	Unilateral right n=14 (24.1%)	Unknown n=10 (17.2%)
Bilateral	6 (46.2%)	9 (42.9%)	4 (28.6%)	4 (40.0%)
Unilateral left	3 (23.1%)	8 (38.1%)	3 (21.4%)	1 (10.0%)
Unilateral right	4 (30.8%)	4 (19.0%)	7 (50.0%)	3 (30.0%)
Unknown	0 (0%)	0 (0%)	0 (0%)	2 (20.0%)

Table 3a. Location analysis of OMA at first surgical recurrence.

First surgery / DIE location at second surgery	DIE					SUP n=27	OMA n=49
	Uterosacral ligament n=3/29	Vagina n=11/29	Intestine n=10/29	Bladder n=1/29	Others* n=4/29		
Uterosacral ligament	0	1 (3.4%)	1 (3.4%)	1 (3.4%)	1 (3.4%)	7 (25.9%)	9 (18.4%)
Vagina	0	8 (27.6%)	5 (17.2%)	0	0	10 (37.0%)	23 (46.9%)
Intestine	1 (3.4%)	4 (13.8%)	4 (13.8%)	0	2 (6.9%)	12 (44.4%)	15 (30.6%)
Bladder	0	0	0	0	0	2 (7.4%)	3 (6.1%)
Others*	2 (6.9%)	1 (3.4%)	1 (3.4%)	0	1 (3.4%)	1 (3.7%)	5 (10.2%)

Table 3b. Location analysis of DIE at recurrent surgery. Note: The 105 patients with DIE at second surgery are analyzed according to the initial lesion subtype. For 29 of them it was DIE, for 27 SUP and for 49 OMA at the first surgery.
*umbilicus, appendix, inguina, round ligament of uterus

Discussion

In the present study, we demonstrate that the time to first recurrent endometriosis surgery is independent from the endometriosis subtype observed at the initial surgery. Moreover, at subsequent surgery the endometriosis subtype observed is likely to be the same subtype observed previously. Interestingly, however, there is a high percentage of patients that present with more severe lesion subtypes, particularly DIE. The trend towards more severe endometriosis subtypes in these patients implies disease progression may occur overtime irrespective of surgical removal.

To the best of our knowledge, this is the first study to compare the time to recurrence between different endometriosis subtypes. The median time to first recurrence for

all women with endometriosis was 31 months, similar to the 30 months reported by Liu et al [25].

It is important to note that our study only included patients who had recurrent endometriosis lesions confirmed through a second surgery. We have specifically selected this cohort because 1) we believe that, given a long enough follow up there is significant potential for all women with endometriosis to recur and thus it becomes difficult to define a non-recurrence group, particularly if surgery is required to confirm the diagnosis and, 2) by including women that have had an initial, complete excision of endometriosis we can confirm that at this point in time these women were devoid of macroscopic endometriosis lesions. This study does not report on women who did not require subsequent surgical intervention, thus it does not describe the likelihood of all endometriosis to recur, but rather only the time to surgical intervention for the group of women with endometriosis that have recurrence significant enough to require additional surgical intervention. Further studies that examine whether there are difference in recurrence in all patients, i.e. those that do not require surgical intervention would be interesting, but challenging to design and perform.

Whether endometriosis represents a progressive disease that worsens over time is not resolved but attracting more attention. A 3-year prospective study suggested that endometriosis is a progressive disease [13] which was supported by a review of adolescence endometriosis [17], with an additional study showing development from peritoneal to ovarian endometriosis, including uterosacral ligament lesions during a 2-5 years follow up [19]. On the contrary, an analysis of randomized control studies (RCT) on adolescents showed 71% of the patients without endometriosis excision did not progress [18]. However, a single RCT contributed the majority of cases to this analysis with a short follow up of only 4 to 6 months [26]. The findings of our study agree with the suggestion of progression and extend them to a broader population with a longer follow up.

OMA was the most common endometriosis subtype that was observed in this group of patients (53.4%). With OMA being the easiest type of endometriosis to diagnose, it seems plausible that they are more usually treated via surgery. If the higher incidence of recurrence is solely due to an observation bias, or that OMA is more likely to recur cannot be answered by the current study. A recent randomized controlled study evaluating levonorgestrel-IUD reported 25-37.5% OMA recurrence [27]. However, recurrent surgery on the ovary is related to ovarian reserve damage [28] and recurrent surgery for endometriosis in general is related to stress, complications as well as personal and social costs. Therefore, caution is required firstly to adhere to the guidelines on the indications for endometriosis surgery and surgical technique and secondly to decrease the risk of recurrence via hormonal suppressive therapy [29-32].

The underlying pathogenesis of endometriosis recurrence is unclear. If recurrence derives from residual endometriotic cells that remain after surgery, or from de novo lesions is a matter of debate [8]. It has been reported that DIE lesions reappeared at subsequent surgery in the same area of the pelvis as at the previous surgery [33], possibly due to the high number of incomplete surgeries included in the study. In another study, 50 out of 62 (80.6%) patients with recurrent endometriomas had recurrence on the treated ovary [34]. Our study also shows that the majority of recurrent endometriomas were on the same ovary, which could indicate residual lesions. However, lesions on the contralateral ovary, or other areas were also observed indicating that de novo lesion development is possible especially since some recurrences were documented after a long nascent period. Another possibility

could be that recurrent lesions at different locations than the initial lesion could occur by endometriotic tissue dissemination during the first surgery.

An important limiting factor in endometriosis research is that although endometriosis recurrence can be well defined within a retrospective study, identifying and confirming non-recurrence is impossible. Thus, no non-recurrent group of patients was included in our study. Many patients may not undergo a subsequent surgery for endometriosis, choosing to tolerate endometriosis-associated symptoms, or to treat the symptoms medically. A detailed follow-up including this information is perhaps possible within a prospective study with long enough follow-up since many recurrences occur after 48 months or even longer. However, due to the difficulty of an accurate non-surgical diagnosis of endometriosis the classification of a patient as non-recurrent would be challenging even in such a prospective follow-up study.

Based on the surgical protocols we assumed in each case that the endometriotic lesions were completely excised. It is however, possible that this was not always the case and thus some subsequent surgeries could be the result of endometriosis persistence instead of endometriosis recurrence. The same applies to the endometriosis subtype with some misdiagnosed at the initial surgery. Moreover, we cannot exclude that selection bias due to the tertiary nature of our clinic was in part a reason for the high proportion of patients with endometriosis progression since less severe endometriosis subtypes may have been surgically treated in other hospitals. However, the tendency of endometriosis progression was observed also on the patients exclusively treated in our clinic. Finally, we lacked reliable data on postoperative hormonal medication. Although there is a consistency of prescription within the single clinic, it is impossible to confirm compliance. We could assume however, that since the time to recurrence was not statistically significantly different between groups there was also no significant difference in the hormonal medication used.

The main advantages of the current study should also be mentioned. It provides real world data and contrary to available studies, a very long follow-up, often including the whole reproductive time, traversing many recurrent surgeries per patient. Moreover, recurrences are well defined and described by laparoscopy, not by imaging alone, adding to the accuracy of the observations. Finally, the inclusion of all hospital-based recurrent patients provides more generalizability compared to the population included in available RCTs primarily designed for other purposes.

The results of the study will help clinicians to better comprehend the evolution of endometriosis in recurrent surgeries and ultimately provide valuable information for patient counselling, especially after surgery.

Supplemental tables and figures

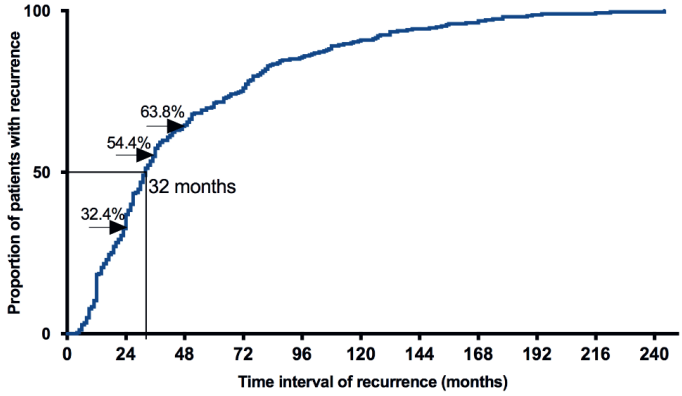

Figure S1. The median time to recurrence is 32 months. The black arrows show the percentage of patients with recurrence at 24 months, 36 and 48 months, respectively.

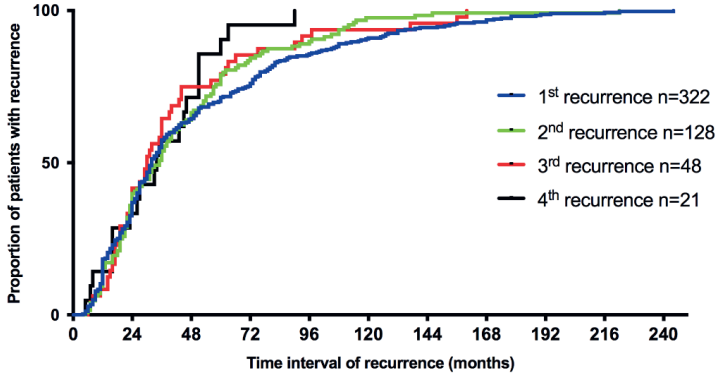

Figure S2. Time to first, second, third and fourth recurrence. The time from the first to second (1st recurrence), second to third (2nd recurrence), third to fourth (3rd recurrence) and fourth to fifth (4th recurrence) surgery is illustrated in different colors. The median time to the first, second, third and fourth recurrent surgery is 32, 35, 30 and 34 months, respectively. No statistically significant difference was observed.

Second surgery / **Third surgery**	SUP (N= 11); median time to recurrence (min-max)	OMA (N= 48); median time to recurrence (min-max)	DIE (N= 32); median time to recurrence (min-max)
SUP	6 (54.5%); 40.5 (9-125)	3 (10.7%); 112 (23-119)	7 (21.9%); 27 (7-61)
OMA	0 (0%)	25 (52.1%); 35 (8-222)	9 (28.1%); 29 (12-89)

DIE	5 (45.5%); 23 (13-70)	17 (35.4%); 43 (6-114)	15 (46.9%); 54 (12-146)
Unknown subtype	0	3 (6.2%); 21 (5-38)	1 (3.1%); 23

Table S1. Evolution of endometriosis from second to third surgery. In each cell, the number of cases evolving into SUP, OMA, DIE or unknown lesion subtype at recurrent surgery and their percentage is given.

Third surgery / **Fourth surgery**	SUP (N= 8); median time to recurrence (min-max)	OMA (N= 15); median time to recurrence (min-max)	DIE (N= 13); median time to recurrence (min-max)
SUP	4 (50%); 29 (11-90)	1 (6.7%); 137	2 (15.4%); 46 (30-62)
OMA	1 (12.5%); 17	9 (60.0%); 17 (6-156)	5 (38.5%); 39 (27-93)
DIE	3 (37.5%); 29 (24-160)	5 (33.3%); 32 (15-63)	6 (46.2%); 40 (24-141)
Unknown	0	0	0

Table S2. Evolution of endometriosis from third to fourth surgery. In each cell, the number of cases evolving into SUP, OMA, DIE or unknown lesion subtype at recurrent surgery and their percentage is given.

References

1. Janssen E.B, Rijkers A.C.M, Hoppenbrouwers K, Meuleman C, D'Hooghe T.M. Prevalence of endometriosis diagnosed by laparoscopy in adolescents with dysmenorrhea or chronic pelvic pain: a systematic review. *Hum Reprod Update* 2013, 19: 570-82.

2. Gylfason J.T, Kristjansson K.A, Sverrisdottir G, Jonsdottir K, Rafnsson V, Geirsson R.T. Pelvic endometriosis diagnosed in an entire nation over 20 years. *Am J Epidemiol* 2010, 172: 237-43.

3. Bougie O, Yap M, Sikora L, Flaxman T, Singh S. Influence of race/ethnicity on prevalence and presentation of endometriosis: A systematic review and meta-analysis. *Bjog* 2019, 126: 1104–1115.

4. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani P.G. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. *Hum Reprod* 2007, 22: 266-71.

5. Simoens S, Hummelshoj L, Dunselman G, Dirksen C, Endocost C.W, D'Hooghe T. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. *Hum Reprod* 2012, 27: 1292-9.

6. Duffy J.M.N, Arambage K, Correa F.J.S, Olive D, Garry R, Barlow D.H, Farquhar C, Jacobson T.Z. Laparoscopic surgery for endometriosis. *Cochrane Database Syst Rev* 2014, 3: Cd011031.

7. Cea Soriano L, López-Garcia E, Schulze-Rath R, Garcia Rodríguez L.A. Incidence, treatment and recurrence of endometriosis in a UK-based population analysis using data from The Health Improvement Network and the Hospital Episode Statistics database. *Eur J Contracept Reprod Health Care* 2017, 22: 334-343.

8. Guo S.W. Recurrence of endometriosis and its control. *Hum Reprod Update* 2009, 15: 441-61.

9. Vercellini P, Crosignani P.G, Abbiati A, Somigliana E, Viganò P, Fedele L. The effect of surgery for symptomatic endometriosis: the other side of the story. *Hum Reprod Update* 2009, 15: 177-88.

10. Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis and adenomyotic nodules of the rectovaginal septum are three different entities. *Fertil Steril* 1997, 68: 11.

11. Cornillie F.J, Oosterlynck D, Lauweryns J.M., Koninckx P.R. Deeply infiltrating pelvic endometriosis: histology and clinical significance. *Fertil Steril* 1990, 53: 978-83.

12. Vercellini P, Trespidi L, De G.O, Cortesi I, Paraxxini F, Crosignani P.G. Endometriosis and pelvic pain: relation to disease stage and localization. *Fertil Steril* 1996, 65: 299-304.

13. Koninckx P.R, Lesaffre E, Meuleman C, Cornillie F.J, Demeyere S. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertil Steril* 1991, 55: 759-65.

14. Chapron C, Fauconnier A, Dubuisson J.B, Barakat H, Marco V, Bréart G. Deep infiltrating endometriosis: relation between severity of dysmenorrhoea and extent of disease. *Hum Reprod* 2003, 18: 760-6.

15. Fauconnier A, Chapron C, Dubuisson J.B, Marco V, Dousset B, Bréart G. Relation between pain symptoms and the anatomic location of deep infiltrating endometriosis. *Fertil Steril* 2002, 78: 719-26.

16. Nirgianakis K, Gasparri L.M, Radan A.P, Villiger A, McKinnon B, Mosimann B, Papadia A, Mueller M.D. Obstetric complications after laparoscopic excision of posterior deep infiltrating endometriosis: a case-control study. *Fertil Steril* 2018. 110(3): p. 459-466.

17. Brosens I, Gordts S, Benagiano G. Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. *Hum Reprod* 2013, 28: 2026-31.

18. Evers J.L.H. Is adolescent endometriosis a progressive disease that needs to be diagnosed and treated? *Hum Reprod* 2013, 28: 2023.

19. Unger C.A, Laufer, M.R. Progression of endometriosis in non-medically managed adolescents: a case series. *J Pediatr Adolesc Gynecol* 2011, 24: e21-3.

20. Audebert A, Lecointre L, Afors K, Koch A, Wattiez A, Akladios C. Adolescent Endometriosis: Report of a Series of 55 Cases With a Focus on Clinical Presentation and Long-Term Issues. *J Minim Invasive Gynecol* 2015, 22: 834-40.

21. Guo S.W, Martin D.C. The perioperative period: a critical yet neglected time window for reducing the recurrence risk of endometriosis? *Hum Reprod* 2019, 34: 1858–1865.

22. von Elm E, Altman D.G, Egger M, Pocock S.J, Gøtzsche P.C. Vandenbroucke J.P. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007, 370: 1453-7.

23. Chapron C, Lafay-Pillet M.C, Monceau E, Borghese B, Ngô C, Souza C, Ziegler D.D. Questioning patients about their adolescent history can identify markers associated with deep infiltrating endometriosis. *Fertil Steril* 2011, 95: 877-81.

24. Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller M.D. Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence. *Acta Obstet Gynecol Scand* 2014, 93: 1262-7.

25. Liu X, Yuan L, Shen F, Zhu Z, Jiang H, Guo S.W. Patterns of and Risk Factors for Recurrence in Women With Ovarian Endometriomas. *Obstet Gynecol* 2007, 109: 10.

26. Harrison R.F, Barry-Kinsella C. Efficacy of medroxyprogesterone treatment in infertile women with endometriosis: a prospective, randomized, placebo-controlled study. *Fertil Steril* 2000, 74: 24-30.

27. Chen Y.J, Hsu T.F, Huang B.S, Tsai H.W, Chang Y.H, Wang P.H. Postoperative maintenance levonorgestrel-releasing intrauterine system and endometrioma recurrence: a randomized controlled study. *Am J Obstet Gynecol* 2017, 216: 582.e1-582.e9.

28. Ferrero S, Scala C, Racca A, Calanni L, Remorgida V, Venturini P.L, Maggiore U.L.R. Second surgery for recurrent unilateral endometriomas and impact on ovarian reserve: a case-control study. *Fertil Steril* 2015, 103: 1236-43.

29. Seracchioli , Mabrouk M, Frascà C, Manuzzi L, Montanari G, Keramyda A, Venturoli S. Long-term cyclic and continuous oral contraceptive therapy and endometrioma recurrence: a randomized controlled trial. *Fertil Steril* 2010, 93: 52-6.

30. Vercellini P, Matteis S.D.E, Somigliana E, Buggio L, Frattaruolo M.P, Fedele L. Long-term adjuvant therapy for the prevention of postoperative endometrioma recurrence: a systematic review and meta-analysis. *Acta Obstet Gynecol Scand* 2013, 92: 8-16.

31. Lee S.R. Yi K.W, Song J.Y, Seo S.K, Lee D.Y, Cho S, Kim S.H. Efficacy and Safety of Long-Term Use of Dienogest in Women With Ovarian Endometrioma. *Reprod Sci* 2018, 25: 341-346.

32. Koga K, Takamura M, Fujii T, Osuga Y. Prevention of the recurrence of symptom and lesions after conservative surgery for endometriosis. *Fertil Steril* 2015, 104: 793-801.

33. Vignali M, Bianchi S, Candiani M, Spadaccini G, Oggioni G, Busacca M. Surgical treatment of deep endometriosis and risk of recurrence. *J Minim Invasive Gynecol* 2005, 12: 508-13.

34. Exacoustos C, Zupi E, Amadio A, Amoroso C, Szabolcs B, Romanini M.E, Arduini D. Recurrence of endometriomas after laparoscopic removal: sonographic and clinical follow-up and indication for second surgery. *J Minim Invasive Gynecol* 2006, 13: 281-8.

Chapter 3

Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence

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Abstract

Objective: To evaluate possible predictive factors for recurrence after laparoscopic segmental bowel resection for bowel endometriosis.

Design: Cohort study

Setting: Academic tertiary referral centre

Methods: A total of 95 symptomatic women with bowel endometriosis who underwent laparoscopic segmental bowel resection at the Endometriosis clinic, University of Bern between 2002 and 2012 were enrolled in this cohort study. Since 14 women were lost to follow-up 81 formed the final cohort. Clinical and histological characteristics were examined as possible predictive factors for disease recurrence.

Main outcome measures: Recurrence, defined as a subsequent operation due to recurrent endometriosis-associated pain with a histologically confirmed endometriotic lesion.

Results: Recurrence was observed in 13 (16%) patients. Variables that were significantly associated to recurrence by the Cox regression analysis were positive bowel resection margins (hazard ratio: 6.5, 95% CI: 1.8-23.5, p: 0.005), age< 31 years (hazard ratio: 5.6, CI: 1.7-18.6, p: 0.005) and BMI≥ 23 kg/m^2 (hazard ratio: 11.0, 95% CI: 2.7-44.6, p: 0.001).

Conclusions: Positive bowel resection margins as well as age<31 years and BMI≥ 23 kg/m^2 appear to be independent predictors of disease recurrence.

Introduction

Bowel endometriosis was first described by Sampson [1] and is characterized by the involvement of the subserosa layer of the bowel [2]. It can present at any level from the anal canal to the small intestine, although it is most frequently located in the rectum and sigmoid colon [2]. It is also typically associated with deep infiltrating endometriosis (DIE) in other locations, such as the ureters and ovaries and has been estimated to affect between 3.8% to 37% of the patients diagnosed with endometriosis [4].

Conservative medical therapy is usually only temporarily effective with symptom recurrence rates as high as 76 % [5]. In these cases surgical excision, which is considered to be effective in relieving pain and improving quality of life [4, 6-9] represents the treatment of choice. The current surgical approach to bowel endometriosis encompasses a number of techniques (bowel shaving, disc resection, segmental bowel resection). It has been reported that a wide margin of excision achieves a lower endometriosis recurrence rate [4, 10]. However, even with segmental bowel resections, recurrences are observed. The recurrence rate varies from study to study and can be up to 20% depending on how the recurrence is defined [11].
The purpose of this study was to examine if certain histological and clinical characteristics are associated with recurrence after laparoscopic segmental bowel resection. Furthermore, the intra- and postoperative complication rate was evaluated.

Methods

This is a retrospective review of a prospectively maintained electronic database of patients with DIE in the rectovaginal septum (RVS) who underwent a laparoscopic operation between May 2002 and May 2012 at the University Hospital of Berne. To be part of the final study population the following criteria had to be satisfied: premenopausal status, symptomatic disease, retention of at least one ovary, a minimum of 12-month follow-up period and treatment via segmental bowel resection. The project was approved by the relevant ethical committee and written informed consent was obtained from all participants prior to surgery. The clinical data (age, BMI, symptoms, previous surgical and medical therapy), operative data and the histological results were analyzed retrospectively.

Before surgery, a detailed medical history was collected and all women had an accurate physical and imaging examination. Couple infertility was defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Indications for surgical intervention included severe, incapacitating, endometriosis-associated symptoms (dysmenorrhea, pelvic pain, deep dyspareunia, dyschezia, dysuria or urinary urgency) not responsive to medical therapy as well as the presence of advanced disease indicated by anatomic distortion of the ureter. The strategy of the operation was to remove all the macroscopically visible endometriotic lesions. All patients were informed in detail about potential complications including the possibility of protective colostomy so that they could choose between a definitive therapy during the first operation, or a second look operation after intra-operative findings were discussed.

All patients likely to require a bowel resection underwent bowel preparation and routinely ad¬ministered intravenous antibiotics before the laparoscopic procedure.

The clinical symptoms as well as the physical and imaging examination indicated preoperatively a possible bowel involvement but the decision for a segmental bowel resection was first made intraoperatively. The RVS was dissected and the nodule was mobilized. Superficial rectal lesions were treated by shaving alone. Deeply infiltrative nodules were treated by either segmental or disc resection, depending on the circumference and length of rectal involvement. In cases with extensive involvement where disc resection was deemed inadequate for macroscopic clearance, segmental resection was performed and the anastomosis was then fashioned by using a double-staple technique. The specimen was retrieved after widening of the suprapubical incision (3-4cm). All anastomoses were routinely air tested. Any air leak was oversewn laparoscopically and retested. Defunctioning ileostomies were performed selectively. The level of the end-to-end anastomosis was defined, according to the distance from the anus, as high/medium (≥8 cm), low (>5 and <8 cm) and ultralow (≤5 cm). Additional procedures were performed during the same operation to remove other localizations of endometriosis. The histological criteria for determining the presence of bowel endometriotic lesions were the presence of ectopic endometrial glands and stroma. The bowel sections were examined to evaluate the vertical infiltration of the endometriotic lesion in the intestinal wall (subserosa, muscularis propria, submucosa, mucosa) and the involvement of the resection margins. Positive resection margins were defined as the presence of endometriosis in one of the tissue rings from the circular stapler or in the margins of the bowel specimens if no tissue rings were available.

The clinical database was analyzed to evaluate the intra- and postoperative complications as well as the disease recurrences. Recurrence was defined as a subsequent operation due to recurrent endometriosis-associated pain with a histologically confirmed endometriotic lesion. This was not necessarily a recurrent endometriotic lesion of the bowel. If the patient did not continue presenting in our clinic, she or her family physician was contacted. In case of recurrence with a subsequent operation in another clinic the operative and histological report were requested. Clinical and histological characteristics were examined as possible predictive factors for disease recurrence.

Statistical analysis

Median values and range were calculated for continuous variables and percentages for the qualitative variables. To investigate the possible predictive factors for recurrence univariate analysis was performed, with the log-rank test applied to survival curves that were obtained with the Kaplan-Meier method. Finally, multivariate analysis has included only those factors that had reached a probability value of <0.1 at univariate analysis and has been carried out with the use of the Cox regression method. The continuous variables, age, BMI and length of resected bowel were converted into binomial variables after finding the best possible cut-off value through a ROC curve analysis. Significance was set at a P value of <0.05. Stata 12 was used for the statistical analysis.

Results

During the 10-year study period, 213 patients underwent laparoscopic surgery for deep infiltrating endometriosis involving the RVS. A laparoscopic segmental bowel resection was completed in 95 of these patients (44.6%). Before surgery, pelvic and abdominal ultrasound was performed. Magnetic resonance imaging and

colonoscopy was performed on 21 (22.1%) and 9 (9.5%) patients, respectively. The clinical characteristics are provided in Table 1.

Age (years), median (range)	33 (24-49)
BMI (Kg/m^2), median (range)	22 (16-32)
Couple infertility, number (%)	27 (28.4)
Nulliparous, number (%)	76 (80.0)
Previous surgery for endometriosis, number (%)	76 (80.0)
Previous hysterectomy, number (%)	8 (8.4)
Previous right Adnexectomy, number (%)	3 (3.2)
Previous left Adnexectomy, number (%)	4 (4.2)
Previous hormone therapy last 3 months before laparoscopy, number (%)	58 (61.1)
Dysuria or urinary urgency, number (%)	4 (4.2)
Dyschezia, number (%)	44 (46.3)
Deep dyspareunia, number (%)	37 (38.9)
Dysmenorrhea or pelvic pain, number (%)	90 (94.7)

Table 1. Patient characteristics (95 patients)

Surgical data

The bowel lesions were located in recto-sigmoid junction, with 8 (8.4 %) cases identified with a second endometriotic lesion in the small intestine that also required treatment (ileocecal junction in four cases and ileal in the other four). The level of the end-to-end anastomosis was high/medium in 41 cases (43.2%), low in 38 cases (40.0%) and ultra-low in 16 cases (16.8%). Eighty-nine (93.7%) procedures were completed laparoscopically. Of the remaining six procedures, one was a planned open procedure and the other five were open conversions.

In most of the cases segmental bowel resection was associated with other concomitant surgical procedures: the associated interventions were partial resection

of posterior vaginal fornix (n=70, 73.7%), resection of the ureterosacral ligament (n=11, 11.6%), right ovarian endometriotic cystectomy (n=26, 27.4%), left ovarial endometriotic cystectomy (n=24, 25.3%), right adnexectomy (n=1, 1.1%), left adnexectomy (n=13, 13.7%), hysterectomy (n=4, 4.2%), bladder resection (n=7, 7.4%), right ureterolysis (n=27, 28.4%), left ureterolysis (n=56, 58.9%), appendectomy (n=5, 5.3%), left uretero-cystoneostomy (n=1, 1.1%) and extended adhesiolysis (n=43, 45.3%). Due to concomitant ureteral endometriosis, five patients (5.3%) suffered from left and one (1.1%) from right ureteral dilatation. In one of the cases the ureterolysis was not enough to treat the problem and an uretero-cystoneostomy was performed. Major complications occurred in 7 (7.4%) patients, including 4 cases (4.2%) with anastomotic leak, 2 (2.1%) with significant intraoperative blood loss (1500ml and 1600ml) requiring trans¬fusion and 1 (1.1%) with a ureteral lesion. One patient with anastomotic leak manifested a rectovaginal fistula three months later and in another case a rectovaginal fistula occurred simultaneously with an anastomotic leak seven days after the operation. In addition, 5 cases (5.3%) with wound infection were observed.

Twelve patients (12.6%) required temporary defunctioning ileostomies. Four of these were secondary to an anastomotic leak and six of them were primarily because of the ultralow level of the anastomosis (≤5 cm from the anus). In one case the patient had previously received pelvic radiotherapy due to Hodgkin's lymphoma and in another one the ileostomy was performed because of simultaneous ileocecal resection and extended adhesiolysis. Restoration of intestinal continuity was performed after a period of 2–12 months in all cases.

Histological data

The median longitudinal length of the resected bowel specimens was 8 cm (range, 3.5 – 26cm.). In all cases, lesions were found extending from the serosa. In 4 (4.2 %), 49 (51.6 %), 31 (32.6%) and 2 (2.1%) specimens, the depth of infiltration of the endometriosis was at the subserosal, muscular, submucosal and mucosal levels, respectively. In 80 cases (84.2%), the bowel resection margins were negative, whereas in the remaining 15 cases (15.8%) they were positive for endometriosis.

Analysis of possible risk factors

Of the 95 patients, 14 (14.7%) were lost to follow-up and 81 (85.3%) formed the final study population. The median follow-up was 53 (range, 12–120) months. Recurrence was observed in 13 (13.7 %) patients requiring a new operation due to endometriosis-associated pain (17-90 months later). Endometriosis or adenomyosis uteri were histologically confirmed in all of them. In 9 patients the subsequent operation showed endometriotic lesions of the peritoneum and/or ovaries and in two in the rectovaginal septum which were laparoscopically excised. In 4 patients the uterus was excised and adenomyosis uteri was confirmed histologically. Only one patient required a new segmental bowel resection (ileumsegment resection, 5cm long). The histological analysis revealed an endometriotic lesion extending to the muscular layer of the bowel.

Univariate survival analysis for comparison of patients with and without recurrence revealed that positive bowel resection margins, BMI ≥23 and age <31 were all significantly associated with disease recurrence (Table 2). Multivariate survival analysis confirmed a significant association of these factors with disease recurrence (Table 3). The Kaplan-Meier curves are presented in Figure 1, 2 and 3.

	Recurrence N=13 (%)	Non Recurrence N=68 (%)	p-value (log rank test)
Positive margins	5 (38.5)	9 (13.2)	<0.05
Infiltration of subserosa	13 (100)	62 (91.2)	NS
Infiltration of muscularis propria	13 (100)	58 (85.3)	NS
Infiltration of submucosa	4 (30.8)	25 (36.7)	NS
Infiltration of mucosa	0 (0)	2 (2.9)	Not estimable
BMI ≥23 kg/m^2	10 (76.9)	24 (35.3)	<0.01
Age <31 years	9 (69.2)	19 (27.9)	<0.05
Length of resected bowel ≥7 cm	7 (53.8)	45 (66.2)	NS
Concomitant Hysterectomy	0 (0)	4 (5.9)	Not estimable
Previous surgery for endometriosis	11 (84.6)	55 (80.9)	NS
Previous hormone therapy last 3 months before laparoscopy	9 (69.2)	42 (61.8)	NS

Table 2. Univariate analysis of predictive factors for recurrence

	Hazard Ratio (95% Confidence Interval)	p-value (log rank test)
Positive margins	6.5 (1.8-23.5)	0.005
BMI ≥23 kg/m^2	11.0 (2.7-44.6)	0.001

Age <31 years	5.6, (1.7-18.6)	0.005

Table 3. Multivariate analysis of predictive factors for recurrence

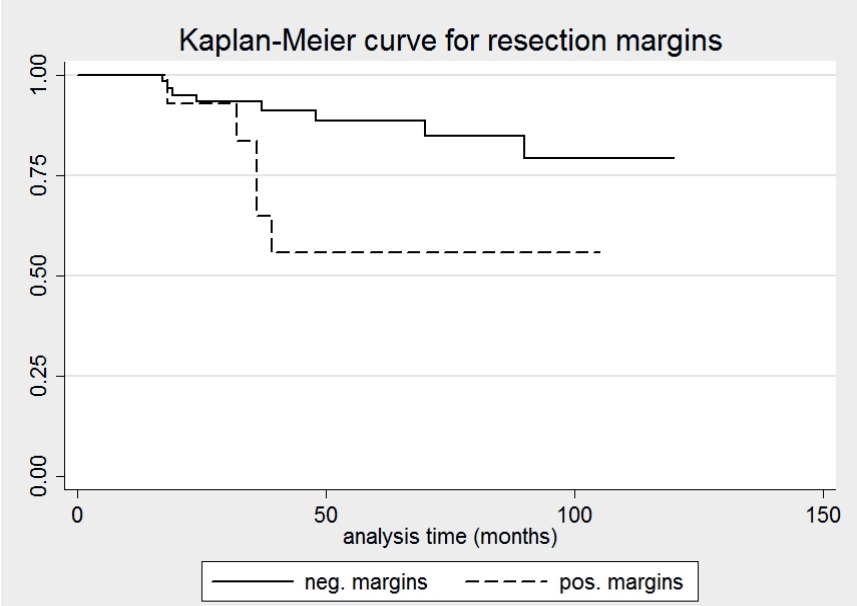

Figure 1. Disease recurrence rate with the Kaplan-Meier method for resection

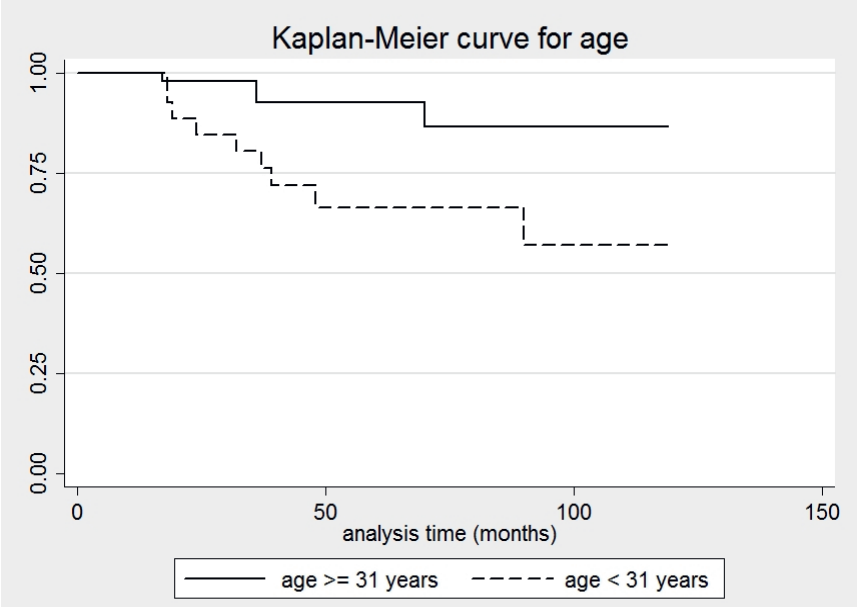

Figure 2. Disease recurrence rate with the Kaplan-Meier method for age.

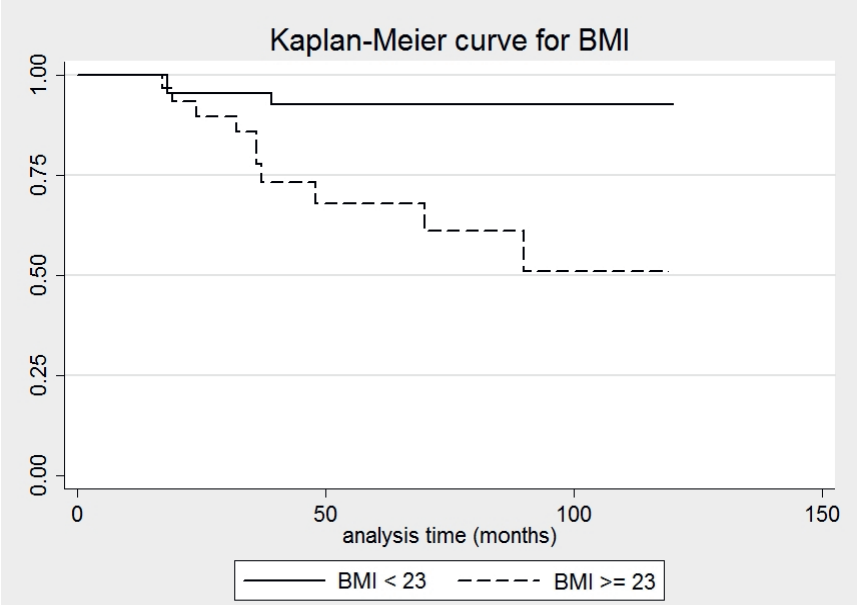

Figure 3. Disease recurrence rate with the Kaplan-Meier method for BMI.

Discussion

To our knowledge, this is the first study to find a correlation between the histopathological margins collected from the resected tissue and the treatment outcomes for bowel endometriosis. Two previous studies have reported no correlation [8, 11]; however, the first study reported that in 66 out of 92 patients a disc resection was performed which in this case cannot be compared with our study population in which disc resections were excluded. In the second study, there was a different outcome measured since only the recurrence of pain but not the subsequent re-operation for endometriosis was examined. More importantly in the previous study the follow up time was limited to a median of 18 months after surgery, whereas most surgeries for recurrence in our study took place only between 17 and 90 months after segmental bowel resection. Moreover, the latter study was possibly, as suggested by the authors, to be underpowered to exclude a possible correlation between the presence of positive margins and the rate of recurrence. Nevertheless, we are aware that our study is limited by its retrospective nature, nor do we have sufficient information to conclude that all patients received similar medical therapy post operation, which may ultimately influence recurrence. It should finally be mentioned that our study population with 81 followed patients is not big enough to allow a detailed evaluation of all different factors that may contribute to disease recurrence.

The fact that younger patients have a higher risk of recurrence is not surprising and has already been presented by other authors [10]. Moreover, the current study, which

shows that high BMI is a predictive factor for recurrence, is in line with the study from Netzhat et al. [12]. Furthermore, it should be noted that none of the 4 patients who had a concomitant hysterectomy presented with recurrence which suggests that hysterectomy is a protective factor for recurrence.

The 15.8% of the bowel specimens that were found with endometriotic lesions in the resection margins in our study is similar to previously published data [11,13]. Bowel endometriotic nodules are usually surrounded by smooth muscle hyperplasia and fibrosis, which may produce mural thickening and luminal stenosis. This macroscopic manifestation is a strong indication for the surgeon to perform the bowel operation. However, not all deep endometriotic bowel nodules are surrounded by extensive fibrosis [14] and in the most of the cases there is a multi-centric involvement [15]. This could be the reason why it is not always possible to achieve a complete excision of the lesions. Some studies suggest that the complete microscopic resection of bowel endometriotic implants might remain impossible, even if large segmental resection is performed [16]. Whether the endometriotic lesion that precipitates the need for a repeat operation is derived from such residual lesions or a new endometriotic lesion is not clear. Anyway, most of the recurrent lesions found in the current study were at other sites but not in the bowel. It may be tempting to suppose that positive bowel resection margins imply an overall more advanced state of disease and thus is indicative of a higher risk of recurrence.

In this study we found the rate of serious complications using the bowel resection technique to treat endometriosis was 4.2% for anastomotic dehiscence and 2.1% for rectovaginal fistula. A recent prospective cohort study [13] found similar complication rates in patients with moderate and severe endometriosis treated either with or without bowel resection. Previous studies have found the rate of intestinal anastomotic leak ranges from 3% to 7% increasing up to 20% for low rectal anastomosis [4] and a rate as high as 10% for rectovaginal fistula [14]. Finally, the temporary ileostomy rate of the current study is similar to previously published data [17].

The question of which surgical technique (bowel shaving, discoid bowel resection, segmental bowel resection) should be favored for the treatment of bowel endometriosis cannot be answered by this study. A crucial factor for the indication of discoid bowel resection seems to be the size of the endometriotic lesion. There is a general agreement that the maximum diameter should not exceed 3cm, with maximum involvement of one-half of the bowel circumference [14, 18]. In these cases it may provide favorable outcomes and low complication rates [19]. This approach was also followed in this study; however, since these cases were excluded from the study population we cannot make any definitive conclusions on this technique.
The results of this study show that the presence of endometriotic tissue in the bowel resection margins as well as age< 31 years and BMI≥ 23 kg/m2 are independent predictors for a subsequent operation for endometriosis or adenomyosis in the future. More frequent gynecological checks or hormonal treatment directly after the operation may be even more important in these cases.

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References:

1. Sampson JA. Intestinal adenomas of endometrial type. Their importance and their relation to ovarian hematomas of endometrial type (Perforating hemorrhagic cysts of the ovary) *Arch Surg* 1922, 5: 217-80.

2. Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V et al. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Hum Reprod* 2003, 18: 157-61.
3. Chapron C, Chopin N, Borghese B, Foulot H, Dousset B, Vacher-Lavenu MC et al. Deeply infiltrating endometriosis: pathogenetic implications of the anatomical distribution. *Hum Reprod* 2006, 21:1839-45.

4. Remorgida V, Ferrero S, Fulcheri E, Ragni N, Martin DC. Bowel endometriosis: presentation, diagnosis, and treatment. *Obstet Gynecol Surv* 2007, 62: 461-70.

5. Shaw RW. Treatment of endometriosis. *Lancet* 1992, 340: 1267-71.

6. Dubernard G, Piketty M, Rouzier R, Houry S, Bazot M, Darai E. Quality of life after laparoscopic colorectal resection for endometriosis. *Hum Reprod* 2006, 21: 1243-7.

7. Duepree HJ, Senagore AJ, Delaney CP, Marcello PW, Brady KM, Falcone T. Laparoscopic resection of deep pelvic endometriosis with rectosigmoid involvement. *J Am Coll Surg* 2002, 195: 754-8.

8. Koh CE, Juszczyk K, Cooper MJ, Solomon MJ. Management of deeply infiltrating endometriosis involving the rectum. *Dis Colon Rectum* 2012, 55: 925-31.

9. Ruffo G, Scopelliti F, Scioscia M, Ceccaroni M, Mainardi P, Minelli L. Laparoscopic colorectal resection for deep infiltrating endometriosis: analysis of 436 cases. *Surg Endosc* 2010, 24: 63-7.

10. Fedele L, Bianchi S, Zanconato G, Bettoni G, Gotsch F. Long-term follow-up after conservative surgery for rectovaginal endometriosis. *Am J Obstet Gynecol* 2004, 190: 1020-4.

11. Mabrouk M, Spagnolo E, Raimondo D, D'Errico A, Caprara G, Malvi D et al. Segmental bowel resection for colorectal endometriosis: is there a correlation between histological pattern and clinical outcomes? *Hum Reprod* 2012, 27: 1314-9.

12. Nezhat C, Hajhosseini B, King LP. Laparoscopic management of bowel endometriosis: predictors of severe disease and recurrence. *JSLS* 2011, 15: 431-8.

13. Meuleman C, Tomassetti C, Wolthuis A, Van Cleynenbreugel B, Laenen A, Penninckx F, Vergote I, D'hoore A, D'hooghe T. Clinical outcome after radical excision of moderate-severe endometriosis with or without bowel resection and reanastomosis: a prospective cohort study. *Ann Surg* 2014, 259: 522-31.

14. Remorgida V, Ragni N, Ferrero S, Anserini P, Torelli P, Fulcheri E. How complete is full thickness disc resection of bowel endometriotic lesions? A prospective surgical and histological study. *Hum Reprod* 2005, 20: 2317-20.

15. Kavallaris A, Kohler C, Kuhne-Heid R, Schneider A. Histopathological extent of rectal invasion by rectovaginal endometriosis. *Hum Reprod* 2003, 18: 1323-7.

16. Anaf V, El Nakadi I, De Moor V, Coppens E, Zalcman M, Noel JC. Anatomic significance of a positive barium enema in deep infiltrating endometriosis of the large bowel. *World J Surg* 2009, 33: 822-7.

17. Ruffo G, Sartori A, Crippa S, Partelli S, Barugola G, Manzoni A, Steinasserer M, Minelli L, Falconi M. Laparoscopic rectal resection for severe endometriosis of the mid and low rectum: technique and operative results. *Surg Endosc*. 2012, 26: 1035-40.

18. Lewis LA, Nezhat C. Laparoscopic treatment of bowel endometriosis. *Surg Technol Int* 2007, 16: 137-41.

19. Fanfani F, Fagotti A, Gagliardi ML, Ruffo G, Ceccaroni M, Scambia G et al. Discoid or segmental rectosigmoid resection for deep infiltrating endometriosis: a case-control study. *Fertil Steril* 2009, 94: 444-9.

Chapter 4

Regression of the inflammatory microenvironment of the peritoneal cavity in women with endometriosis by GnRHa treatment.

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Abstract

Objective: To investigate the effect of gonadotropin-releasing hormone analogues (GnRHa) on the peritoneal fluid microenvironment in women with endometriosis.

Study design: Peritoneal fluid was collected from 85 women with severe endometriosis (rAFS stage III and IV) during laparoscopic surgery during the proliferative phase. Prior to surgery clinical data were collected. The concentrations of specific markers for endometriosis in the peritoneal fluid were determined using an ELISA and a comparison between peritoneal fluid markers in women using GnRHa and no hormonal treatment was performed using a non-parametric Mann-Whitney U test.

Results: The study included peritoneal fluid from 39 patients who had been administered GnRHa (Zoladex(®)) in the three months prior to surgery and 46 from women with no hormonal treatment in this period. Concentrations of IL-8, PAPP-A, glycodelin-A and midkine were significantly reduced in the GnRHa treatment group compared to women receiving no hormonal treatment. RANTES, MCP-1, ENA-78, TNF-α, OPG, IP-10 and defensin showed no significant change between the two groups.

Conclusions: GnRHa mediate a significant regression in the inflammatory nature of the peritoneal microenvironment in women with endometriosis.

Introduction

Endometriosis is an extremely prevalent estrogen-dependent gynecological disorder affecting at least 10% of reproductive-aged women worldwide [1]. It is characterized by the growth of endometrial tissue outside the uterine cavity and can result in severe pelvic pain [2] and subfertility [3].

Currently the most widely accepted theory of endometriosis etiology purports that retrograde menstruation allows the implantation of viable endometrial cells at ectopic sites [4]. The ectopic lesions then secrete chemotactic molecules recruiting immune cells to the peritoneal fluid (PF) and these in turn secrete pro-inflammatory cytokines, further stimulating the proliferation of the lesion [5]. Elevated concentrations of numerous molecules have been reported in the peritoneal fluid of women with endometriosis [6-9]. In addition, interleukin-6 (IL-6), tumour necrosis factor-a (TNF-a), regulated on activation normal T cell expressed and secreted (RANTES), osteoprotegerin (OPG) and glycodelin have also been associated with dysmenorrhoea in endometriosis [10-12].

GnRH analogues (GnRHa) are an important treatment modality for endometriosis, significantly reducing endometriosis-related pain [13]. Current knowledge suggests this is mediated primarily by the induction of a hypo-estrogenic state. The exact role of hypoestrogenism, however, is not well defined, as the responsiveness of endometriotic lesions to estrogens remains unclear. Estrogen receptors (ER) have been reported in endometriotic lesions [14] although at lower expression in ectopic tissue than matching eutopic tissue [15] and with no cyclical variations [16], and the responsiveness of endometriosis tissue to ovarian steroids differs significantly between eutopic and ectopic tissue in the same patients [17]. GnRHa may also act locally. This activity could be mediated both indirectly by ER receptors present on peritoneal macrophages [18], or directly via GnRH receptors (GnRHR) on the endometriotic lesions themselves [19]. Previous evidence suggests GnRHa modulate the peritoneal microenvironment by decreasing the concentrations of some angiogenic and growth factors, as well as cytokines [20].

Given the importance of GnRHa use in the treatment of endometriosis we wished to extend the limited knowledge on what effect these treatments have on the local peritoneal microenvironment. We therefore analysed the concentration of a number of molecules found upregulated in the peritoneal fluid of women with endometriosis, and compared these concentrations in a case–control study between women with and without GnRHa treatment prior to surgery.

Materials and Methods

Sample and data collection

Women undergoing laparoscopic procedures for endometriosis in the Department of Obstetrics and Gynaecology, University of Berne (Switzerland) between 2007 and 2011 were recruited for the study. Written informed consent and detailed information on hormonal treatment usage was obtained from all participants prior to surgery. Patients using hormonal contraception (combined or gestagen-only) or vaginal progesterone the last three months prior to surgery were excluded from the study. Moreover, patients using any anti-inflammatory drugs or suffering from

other inflammatory diseases were excluded. Only patients with severe endometriosis (rAFS stage 3 and 4) were included in the study.

The project was approved by the relevant Ethical Committee and all procedures were performed during the proliferative phase of the menstrual cycle.

Peritoneal fluid was aspirated from the pouch of Douglas and clarified by centrifugation (10 min at 1800 x g). Peritoneal fluid samples were stored undiluted in sterile cryotubes at -70°C for up to five years prior to assay. Total protein concentration was determined with the micro-bicinchoninic acid method (Quanti-Pro1 BCA, Sigma–Aldrich, St. Louis, USA) [15] on microtitre plates in order to exclude samples that had been accidentally diluted with flush medium during the laparoscopic procedure.

Measurement of cytokine concentrations

Cytokine concentrations were determined by microplate ELISA in duplicate (single for some commercial assay kits). IL-8, TNF-a, RANTES, midkine, OPG, pregnancy-associated plasma protein A (PAPP-A), and glycodelin (PP14) were determined in the PF samples as described in previous reports from our laboratory [6,11]. MCP-1 and IP-10 were determined using the Duo-Set method from R&D Systems (Abingdon, England) using a PF dilution of 1:3 in phosphate- buffered saline containing 1% (w/v) bovine serum albumin. ENA-78 was measured with the commercial ELSIA kit (Quantikine, also from R&D Systems England); in order to increase the sensitivity to 2.5 pg/mL the ratio between sample and assay diluent was increased from 1:5 to 2:5. Neutrophil defensins (combined alpha defensins HNP-1, -2 and -3) were determined with the assay from Hycult Biotech (Uden, the Netherlands). Functional sensitivity of this assay was below 150 pg/mL. PF samples were diluted 1:5 in the supplied dilution buffer. For the commercial assays the protocols supplied by the manufacturers were followed, with the modification that antibody incubations were performed at a controlled temperature of 28°C in a dry incubator/shaker. At the time of these measurements the laboratory had no knowledge of the presence or absence of GnRHa treatment. Statistical analysis for the comparison of marker concentrations between groups (GnRHa treated vs. non-treated) was done by Mann–Whitney U test with the statistical program Graphpad Prism version 3.03. A P value below 0.05 was considered as significant for all tests.

Results

Patients and sample data

Eighty-five women (age 32.7 ± 5.0 years; BMI 23.0 ± 4.3 kg/m^2, mean ± SD) with histologically confirmed endometriosis were included in the study. A total of 39 patients had been taking GnRH agonist medication (Zoladex, 3.6 mg subcutaneously every 28 days) during the three months preceding surgery. The remaining 46 women that made up the control group did not receive any medication. No significant difference in either age or BMI was observed between the treatment or control groups. Patient data and peritoneal fluid characteristics are given in Table 1.

	Non-treated	GnRHa treated	P value
Number of women	46	39	
Age (years)	32.9 ± 4.6	32.4 ± 5.4	0.6778
Body mass index (BMI, kg/m2)	23.7 ± 4.5	22.3 ± 4.1	0.1405
Peritoneal fluid (PF) collected (mL)	11.2 _ 8.2	7.7 ± 5.6	0.0228
Total protein in PF (mg/mL)	33.8 ± 10.1	35.7 ± 6.8	0.3156

Table 1. Demographic data of the endometriosis patients (N = 85), peritoneal fluid characteristics and pain levels in the non-treated and the GnRH agonist treated group.

Peritoneal fluid cytokine concentrations with and without GnRHa treatment

Amongst the studied markers IL-8 (Fig. 1A), pregnancyassociated plasma protein A (PAPP-A, Fig. 2A), glycodelin A (Fig. 2B) and midkine were found to have a significantly reduced concentration in the GnRHa treatment group compared to the control group. In statistical terms the strongest decreases were observed for PAPP-A and glycodelin A ($P < 0.0001$). Other analytes (RANTES, MCP-1, ENA-78, and defensins) showed decreases to similar extents but without reaching statistical significance. In spite of using modified incubation volumes in the assay for ENA-78 (Fig. 1B), 22% and 26% of the obtained values were below the increased detection limit of 2.5 pg/mL for the control and the GnRHa treatment group, respectively. The medians of TNF-a and OPG did not differ between the two groups, and IP-10 was increased in the GnRHa treatment group, but without reaching statistical significance. The results are shown in Table 2.

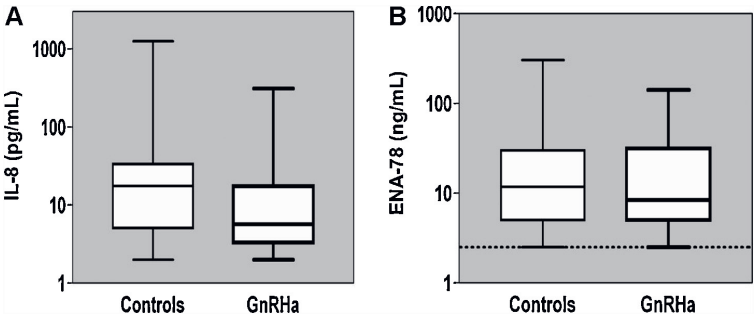

Figure 1. Box-and-whisker plots for the concentrations of IL-8 and ENA-78 in the peritoneal fluid of endometriosis patients treated with GnRH analogues (N = 39, bold print) or not (N = 46, fine print). Boxes represent the median with 25th and 75th percentiles, the whiskers the minimum and the maximum. (A) IL-8; (B) ENA-78. Please note the logarithmic scales and the large variations, as the values mostly range over several orders of magnitude. The dotted line in (B) (ENA-78) represents the detection limit of the assay.

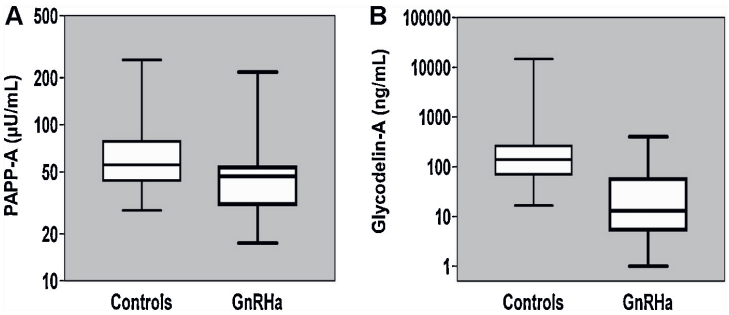

Figure 2. Box-and-whisker plots for A: PAPP-A and B: glycodelin; for details see Fig. 1

Cytokine/marker (Concentration unit)	**Non-treated** (N = 46)	**GnRHa treated** (N = 39)	**P value**
IL-8 (pg/mL)	17.55 (<2–1254)	5.70 (<2–312.4)	**0.0027**
ENA-78 (pg/mL)	11.8 (<2.5–305)	8.4 (<2.5–142)	0.5358
TNF-a (pg/mL)	0.895 (<0.1–6.54)	1.36 (<0.1–20.95)	0.2183
RANTES (pg/mL)	48.8 (<0.2–3527)	36.0 (<0.2–1471)	0.7375
OPG (pg/mL)	3575 (<5–47,900)	3414 (237–43,800)	0.9473
Midkine (ng/mL)	6.91 (0.47–234)	3.96 (<0.1–202)	**0.0111**
MCP-1 (ng/mL)	335 (<20–1202)	236 (<20–4061)	0.1252
IP-10 (pg/mL)	295 (<10–4255)	422 (62–4673)	0.1889
Defensins HNP1–3 (ng/mL)	2.38 (1.01–289)	2.14 (0.83–8.83)	0.1827
PAPP-A (mIU/mL)	55.6 (28.3–260)	46.8 (17.5–217.3)	**0.0006**

Glycodelin (ng/mL)	A	139.4 (16.6–14,600)	13.0 (1.0–402)	**<0.0001**

Table 2. Concentrations of cytokines and other markers in the peritoneal fluid of endometriosis patient groups treated with GnRHa (N = 39) or not (N = 46). Values are median and range. Statistically significant differences between the two groups are printed in bold (P values).

Discussion

GnRHa are a widely used treatment for endometriosis. The success of these compounds in reducing endometriotic-related pain is well known, although due to their hormonal nature they have many unwanted side effects. GnRHa are able to work both on the hypothalamic axis creating a hypo-estrogenic state and directly on extra-pituitary tissue such as the endometrium and the ovary [21-23]. In this case–control study we have found that GnRHa effect the paracrine environment of the peritoneal fluid (PF) and significantly alter the peritoneal fluid microenvironment of women with endometriosis.

Patients using any anti-inflammatory drugs or suffering from other inflammatory diseases were excluded from the study. Nonsteroidal anti-inflammatory drugs could not be excluded, however, since every patient suffered from pain and used some type of analgesic. In our opinion, if skewed to either group the use of these analgesics would have primarily occurred in women without GnRHa prior to surgery. If this were the case, any consequence on the inhibition of inflammation would more likely reduce the cytokine levels in the control group. That could mean that the differences in the results could be even more significant if the patients had not used any non-steroidal anti-inflammatory drugs.

The volume of collected peritoneal fluid was significantly less in the GnRHa group. It is interesting to note that if the total amounts of the molecules are compared (multiplication of our observed concentrations with the volume), the differences between cases and controls become even more significant. Concentration, however, is a more widely used measure to account for variations that inherently occur amongst individuals, without taking the total mass into account. The production of the fluid (solute) is not the same as that of a biologically active molecule.

IL-8, PAPP-A, glycodelin and midkine concentrations were all significantly lower in GnRHa treated women compared to the control group. IL-8 is a chemotactic protein for neutrophils and T lymphocytes and is a potent angiogenic agent [24]. An increased expression of IL-8 has been previously recorded in a number of women with endometriosis [8, 25] and its production has been identified in macrophages, cultured epithelial and stromal cells from ectopic lesions [26] as well as mesothelial cells [25].

We also found a significant decrease in two glycoproteins after GnRHa treatment. PAPP-A and glycodelin are glycoproteins originally identified in the placenta [27] but have since been observed in other reproductive tissue including the endometrium [28, 29]. Although the role of PAPP-A in endometriosis is not clear, peritoneal fluid

concentrations are significantly increased in women with endometriosis [6, 7] and the results of this study show that treatment with GnRHa significantly reduces these concentrations. Glycodelin is predominantly produced by epithelial cells of the endometrium and is thus found in higher concentrations in the endometrium during the secretory phase [30]. Increased concentrations of glycodelin have been observed in both the plasma and the serum of women with deep infiltrating endometriosis (DIE) [31], and a correlation between pain symptoms and glycodelin has been reported [10, 11].

Midkine is a multifunction growth factor strongly induced during oncogenesis and inflammation [32]. Previous studies have shown midkine concentrations are increased in the peritoneal fluid of women with endometriosis, but they can be significantly inhibited by the use of GnRHa [33], a finding that was confirmed by our study. Previous studies have suggested the increased concentrations of midkine in the PF of women with endometriosis may contribute to both the proliferation as well as the adhesion of refluxed endometrial cells to the mesothelial cells of the peritoneal wall [34] Therefore the GnRHa-mediated reduction of this growth factor could contribute to the anti-proliferative effects observed for GnRHa use.

While a significant variation was observed in a number of the molecules we examined we found no significant change in RANTES, MCP-1, ENA-78, TNF-a, OPG or IP-10. These results therefore suggest that while these cytokines may be important in the pathogenesis of endometriosis they might not be related to the reduction in pain and potentially the reduction in lesion size that may occur with GnRHa use. It is also possible that we were unable to identify a significant variation because of our sample size. We believe, however, that splitting 85 patients into the two-group design has given this study an acceptable power to detect real variations. The ability of GnRHa to reduce the pain experienced by endometriosis sufferers has been well documented. Whether GnRHa also reduce the size of endometriotic lesions, however, is less definitive. Studies have shown GnRHa treatment reduced the size of endometriotic lesions in rat models [35] and inhibited the proliferation of cultured ectopic endometrial cells [19, 21], although conversely a recent study found no anti-proliferative effect of GnRHa on endometriotic stromal cells [36]. Importantly however, no definitive in vivo study has been performed in humans as yet to confirm the ability of GnRHa to reduce the size of endometriotic lesions. Whether the reduction in pain, and potentially the reduction in size, mediated by GnRHa use are via the hypo-estrogenic state or via direct local effects is not clear and is not answered by this study.

The results of this study show that the use of GnRHa is capable of mediating a significant regression of the inflammatory microenvironment of endometriotic lesions. By significantly reducing inflammatory cytokines and growth factors in the peritoneal cavity the use of GnRHa may contribute to both the reduction of endometriotic lesion size and pain relief in more ways than just the induction of a hypo-estrogenic state. Directly targeting some of these factors with non-hormonal treatments may achieve the same anti-inflammatory effect while avoiding the significant side effects associated with hormonal treatment.

References

1. Eskenazi B, Warner ML. Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 1997, 24: 235–58.

2. Evans S, Moalem-Taylor G, Tracey DJ. Pain and endometriosis. *Pain* 2007, 132(Suppl. 1): S22–5.

3. D'Hooghe TM, Debrock S, Hill JA, Meuleman C. Endometriosis and subfertility: is the relationship resolved. *Semin Reprod Med* 2003, 21: 243–54.

4. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet Gynecol* 1984, 64: 151–4.

5. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. *Fertil Steril* 2001, 75: 1–10.

6. Bersinger NA, von Roten S, Wunder DM, Raio L, Dreher E, Mueller MD. PAPP-A and osteoprotegerin, together with interleukin-8 and RANTES, are elevated in the peritoneal fluid of women with endometriosis. *Am J Obstet Gynecol* 2006, 195: 103–8.

7. Arici A, Matalliotakis I, Goumenou A, Koumantakis G, Fragouli Y, Mahutte NG. Increased pregnancy-associated plasma protein-A (PAPP-A) concentrations in peritoneal fluid of women with endometriosis. *Am J Reprod Immunol* 2003, 49: 70–4.

8. Ryan IP, Tseng JF, Schriock ED, Khorram O, Landers DV, Taylor RN. Interleukin-8 concentrations are elevated in peritoneal fluid of women with endometriosis. *Fertil Steril* 1995, 63: 929–32.

9. Khorram O, Taylor RN, Ryan IP, Schall TJ, Landers DV. Peritoneal fluid concentrations of the cytokine RANTES correlate with the severity of endometriosis. *Am J Obstet Gynecol* 1993, 169: 1545–9.

10. Bersinger NA, McKinnon B, Kuhn A, Santi A, Mueller MD. Pain symptoms and peritoneal fluid cytokine and marker concentrations in women with and without endometriosis. *J Endometriosis Pelvic Pain Disord* 2009, 1: 137–49.

11. Scholl B, Bersinger NA, Kuhn A, Mueller MD. Correlation between symptoms of pain and peritoneal fluid inflammatory cytokine concentrations in endometriosis. *Gynecol Endocrinol* 2009, 25: 701–6.

12. Velasco I, Acien P, Campos A, Acien MI, Ruiz-Macia E. Interleukin-6 and other soluble factors in peritoneal fluid and endometriomas and their relation to pain and aromatase expression. *J Reprod Immunol* 2010, 84: 199–205.

13. Brown J, Pan A, Hart RJ. Gonadotrophin-releasing hormone analogues for pain associated with endometriosis. *Cochrane Database Syst Rev* 2010. CD008475.

14. Janne O, Kauppila A, Kokko E, Lantto T, Ronnberg L, Vihko R. Estrogen and progestin receptors in endometriosis lesions: comparison with endometrial tissue. *Am J Obstet Gynecol* 1981, 141: 562–6.

15. Nisolle M, Casanas-Roux F, Wyns C, de Menten Y, Mathieu PE, Donnez J. Immunohistochemical analysis of estrogen and progesterone receptors in endometrium and peritoneal endometriosis: a new quantitative method. *Fertil Steril* 1994, 62: 751–9.

16. Lessey BA, Metzger DA, Haney AF, McCarty Jr KS. Immunohistochemical analysis of estrogen and progesterone receptors in endometriosis: comparison with normal endometrium during the menstrual cycle and the effect of medical therapy. *Fertil Steril* 1989, 51: 409–15.

17. Prentice A, Randall BJ, Weddell A, et al. Ovarian steroid receptor expression in endometriosis and in two potential parent epithelia: endometrium and peritoneal mesothelium. *Hum Reprod* 1992, 7: 1318–25.

18. Khan KN, Masuzaki H, Fujishita A, et al. Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. *Hum Reprod* 2005, 20: 2004–13.

19. Khan KN, Kitajima M, Hiraki K, et al. Cell proliferation effect of GnRH agonist on pathological lesions of women with endometriosis, adenomyosis and uterine myoma. *Hum Reprod* 2010, 25: 2878–90.

20. Kupker W, Schultze-Mosgau A, Diedrich K. Paracrine changes in the peritoneal environment of women with endometriosis. *Hum Reprod Update* 1998, 4: 719–23.

21. Borroni R, Di Blasio AM, Gaffuri B, Santorsola R, Busacca M, Vigano P, et al. Expression of GnRH receptor gene in human ectopic endometrial cells and inhibition of their proliferation by leuprolide acetate. *Mol Cell Endocrinol* 2000, 159: 37–43.

22. Tesone M, Bilotas M, Baranao RI, Meresman G. The role of GnRH analogues in endometriosis-associated apoptosis and angiogenesis. *Gynecol Obstet Invest* 2008, 66(Suppl. 1): 10–8.

23. Kang SK, Choi KC, Cheng KW, Nathwani PS, Auersperg N, Leung PC. Role of gonadotropin-releasing hormone as an autocrine growth factor in human ovarian surface epithelium. *Endocrinology* 2000, 141: 72–80.

24. Arici A. Local cytokines in endometrial tissue: the role of interleukin-8 in the pathogenesis of endometriosis. Ann N Y Acad Sci 2002, 955: 101–9 [discussion 18, 396–406].

25. Arici A, Tazuke SI, Attar E, Kliman HJ, Olive DL. Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. *Mol Hum Reprod* 1996, 2:40–5.

26. Shi YL, Luo XZ, Zhu XY, Hua KQ, Zhu Y, Li DJ. Effects of combined 17betaestradiol with TCDD on secretion of chemokine IL-8 and expression of its receptor CXCR1 in endometriotic focus-associated cells in co-culture. *Hum Reprod* 2006, 21: 870–9.

27. Bersinger NA, Altermatt HJ, Birkhauser MH, et al. Non-placental production of pregnancy-associated plasma protein A (PAPP-A): old and new evidence. *Early Pregnancy* 1997, 3: 96–101.

28. Overgaard MT, Oxvig C, Christiansen M, et al. Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein: expression in human reproductive and nonreproductive tissues. *Biol Reprod* 1999, 61: 1083–9.

29. Rutanen EM, Koistinen R, Seppala M, Julkunen M, Suikkari AM, Huhtala ML. Progesterone-associated proteins PP12 and PP14 in the human endometrium. *J Steroid Biochem* 1987, 27: 25–31.

30. Seppala M, Taylor RN, Koistinen H, Koistinen R, Milgrom E. Glycodelin: a major lipocalin protein of the reproductive axis with diverse actions in cell recognition and differentiation. *Endocr Rev* 2002, 23: 401–30.

31. Koninckx PR, Riittinen L, Seppala M, Cornillie FJ. CA-125 and placental protein 14 concentrations in plasma and peritoneal fluid of women with deeply infiltrating pelvic endometriosis. *Fertil Steril* 1992, 57: 523–30.

32. Muramatsu T. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. *J Biochem* 2002, 132: 359–71.

33. Hirota Y, Osuga Y, Koga K, et al. Possible implication of midkine in the development of endometriosis. *Hum Reprod* 2005, 20: 1084–9.

34. Inoh K, Muramatsu H, Ochiai K, Torii S, Midkine Muramatsu T. A heparinbinding cytokine, plays key roles in intraperitoneal adhesions. *Biochem Biophys Res Commun* 2004, 317: 108–13.

35. Altintas D, Kokcu A, Tosun M, Cetinkaya MB, Kandemir B. Comparison of the effects of cetrorelix, a GnRH antagonist, and leuprolide, a GnRH agonist, on experimental endometriosis. *J Obstet Gynaecol Res* 2008, 34: 1014–9.

36. Taniguchi F, Higaki H, Azuma Y, et al. Gonadotropin-releasing hormone analogues reduce the proliferation of endometrial stromal cells but not endometriotic cells. *Gynecol Obstet Invest* 2013, 75: 9–15.

Chapter 5

Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis

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Abstract

Introduction: Recent evidence suggests that assisted reproductive technology (ART) increases the risk of adverse pregnancy outcomes, including placental disorders. Similarly, endometriosis resulted detrimental on placenta previa. However, up to 50% of women with endometriosis suffer from infertility, thus requiring ART. The aim of our metanalysis is to compare women with and without endometriosis undergoing ART in terms of placenta disorders events, to establish if ART itself or endometriosis, as an indication to ART, increases the risk of placenta previa.

Methods: Literature searches were conducted in January 2018 using electronic databases (PubMed, Medline, Scopus, Embase, Science Direct, and the Cochrane Library Scopus). Series comparing pregnancy outcome after ART in women with and without endometriosis were screened and data on placenta previa and placental abruption were extracted.

Results: Five retrospective case–control studies met the inclusion criteria. The meta-analysis revealed that endometriosis is associated with an increased risk of placenta previa in pregnancies achieved through ART (OR 2.96 (95% CI 1.25–7.03); p = 0.01, I^2 =69%, random-effect model). No differences in placental abruption incidence were found (OR 0.44 (95% CI 0.10–1.87); p = 0.26, I^2 = 0%, fixed-effect model).

Conclusion: Patients with endometriosis undergoing ART may have additional risk of placenta previa. Despite the inability to determine if endometriosis alone or endometriosis plus ART increase the risk, physicians should be aware of the potential additional risk that endometriosis patients undergoing ART harbor.

Introduction

Endometriosis is a benign chronic condition affecting approximately 10% of women worldwide [1, 2]. Up to 50% of women with infertility are affected by endometriosis [2]. With such strong association with infertility, the affected patients often require assisted reproductive technology (ART) to conceive. Pregnancies achieved through ART have a higher prevalence of adverse perinatal outcomes compared with those achieved naturally, such as preterm delivery, low birth weight and small for gestational age (American College of Obstetricians and Gynecologists bulletin 671) [3]. Recently, a meta-analysis showed that ART procedures are a risk factor for placenta previa [4].

Although the influence of pregnancy on endometriosis is historically accepted [5], the impact of endometriosis on pregnancy remains controversial [6-23]. Some authors suggest that endometriosis may be responsible for an increased incidence of obstetric complications [24-26]. Women with endometriosis have functional endometrial-like tissue out- side the uterus as well as an aberrant endometrial environment. The inflammatory and metabolic environment associated with endometriosis affects the endometrial receptivity, decidualization and remodeling of the uterine spiral vessels after embryo implantation [27]. The deregulated endometrial receptivity of women affected by endometriosis is associated with progesterone resistance and inadequate uterine contractility. The impaired decidualization in women with endometriosis may result from the abnormal interplay of transcriptional factors, cytokines, and signaling pathways. The inflammatory mediators, oxidative stress and alterations in the uterine junctional zone of patients with endometriosis lead to an abnormal conversion of the uterine spiral arteries into uteroplacental vessels. These subsequent suboptimal endometrial functions, defective decidualization, and pathological vascularization may be responsible for an increase in pregnancy complications, including placental disorders.

Whether ART itself or the underlying reproductive disorder (endometriosis) underpinning ART is responsible for an increase in placental disorders and, therefore, predisposes a poor pregnancy outcome, remains an unanswered question. The aim of this meta-analysis is to compare the incidence of placental disorders in women with and without endometriosis achieving pregnancy through ART, thereby shedding light on this issue.

Methods

Data identification and selection

This meta-analysis was performed in accordance with the Preferred Reporting Items for Systematic reviews and Meta- Analyses (PRISMA) statement. On January 2018, a systematic literature search was carried out. All eligible studies were included without restriction on publication year. Papers were identified using the electronic databases (PubMed, Medline, Scopus, Embase, Science Direct, and the Cochrane Library) using the search terms “assisted reproductive technology” and “endometriosis” and “adverse pregnancy outcome” and “placental disorders” and “placenta previa” and “placental; abruption”. All English-language original reports evaluating the incidence of placenta previa and placental abruption in pregnant women with and without endome- triosis were included. Only studies reporting

pregnancies achieved through ART, including in vitro fertilization (IVF) and intra-cytoplasmatic sperm injection (ICSI) were assessed. Only studies comparing pregnant women with and without endometriosis were included in the meta-analysis. To reduce selection biases, and all the studies were matched for at least three of the following factors: first pregnancy, singleton pregnancy, smoking status and maternal age. Reference lists of already published reviews and original reports were also analyzed to identify potential studies. A diagnosis of endometriosis by US and/or MRI, or histology was accepted. Review articles, case reports, video articles and letters were excluded.

Outcomes

The outcomes considered in our study were placenta pre- via (PP) and placental abruption (PA). Placenta previa was defined as the pathologic condition in which the placenta completely or partially covered the internal cervical os; PA was defined as the pathological separation of the placenta from its site of implantation prior to delivery.

Statistical analysis

The PP and PA events after ART in women with endo- metriosis compared with women without endometriosis were stratified by studies. Pooled odds ratio (OR) or risk ratio (RR) were calculated using fixed- or a random-effects models. The I^2 value was used to quantify the inconsistency across studies. It was calculated to describe the proportion variability in effect estimating resulting from heterogeneity rather than sampling error. A naive cate- gorisation of values for I2 would not be appropriate for all circumstances, although we would tentatively assign adjectives of I^2 as follows: I^2 value ranking from between 0 to 40% was not relevant, 30–60% represented moderate heterogeneity, 50–90% represented substantial heterogeneity, and 75–100% considerable heterogeneity. Graphical representation of each study and pooled analysis are dis- played by forest plots. The contribution of each study in the meta-analysis is graphically reported by squares of different sizes. Confidence intervals (CIs) for each study are presented as a horizontal line passing through the square. The pooled OR or RR are shown as a lozenge in the forest plot where the size corresponds to the 95% CI of the OR or RR. A p value of ≤ 0.05 was considered significant. Statistical analysis was performed using Review Manager 5.3 (http://www.cochrane.org).

Results

Five studies met the inclusion criteria (Fig. 1) and were evaluated [9,11,16,19,26]. A total of 8007 patients undergoing ART were included, of which 1719 (21%) had a diagnosis of endometriosis. Table 1 summarizes the characteristics of the included studies.

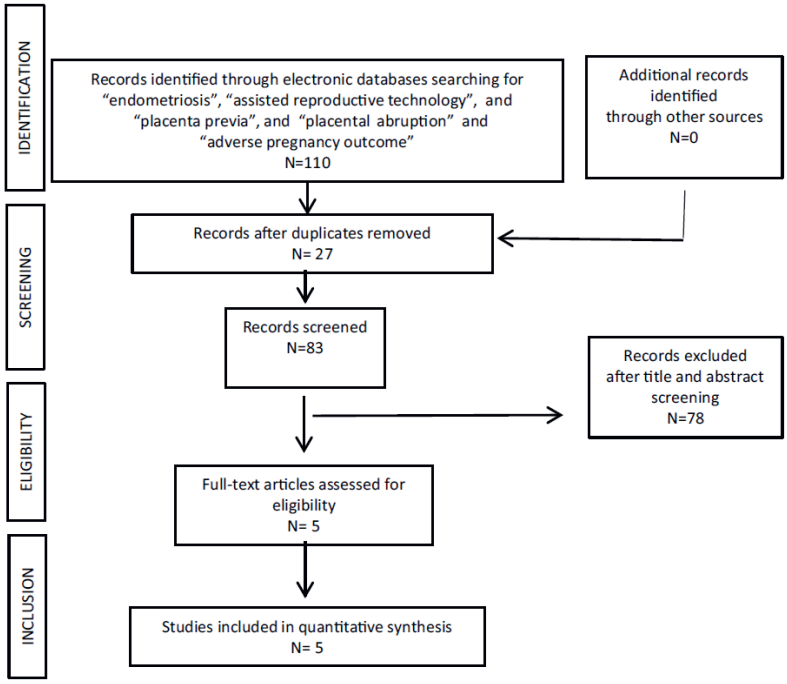

Figure 1. PRISMA flow diagram on the meta-analysis process

References	Study design	ART center	Number of participants		Study group diagnostic procedures (%)	Endometriosis site and/or ASRM stage	Nulliparas (%)			Outcomes considered
			Case study	Control group			Case study	Control group	*p* value	
Healy et al.[28]	Multicentric retrospective cohort study	Melbourne University, Australia Monash University, Australia	1265	5465	n/a	n/a	5253/6730 (78)[a]			APH, PP, PA, PPH
Kuivasaari-Pirinen et al.[16]	Retrospective case–control study	University Hospital of Kuopio, Finland	49	206[b]	The etiology of infertility was defined by laparoscopy,	n/a	41 (83.7)	152 (73.8)	*p* > 0.05	PE, PP, PA, PTD, GDM, SGA, NICU

					ultrasonography, and laboratory parameters (when appropriate); unclear what kind of surgery was performed					
Takemura et al.[23]	Retrospective case–control study	University of Tokyo Hospital, Tokyo, Japan	53	265	Endometriosis was diagnosed by histopathology on surgery (47/53) or MRI (6/53); unclear what kind of surgery was performed	n/a	The parity has been only compared between women with and without placenta previa ($p > 0.05$). Data on parity in patients with and without endometriosis cannot be extracted			PP
Benaglia et al.[7]	Retrospective case–control study	Fondazione Cà Granda, Ospedale Maggiore Policlinico of Milan, Italy	239	239	History of surgery for endometriosis in 186 (78%) patients. Sonographic diagnosis of ovarian endometriosis in 53 (22%) patients	DIE: 77 (41%) Ovarian: 134 (72%)	216 (90)	202 (84)	$p = 0.07$	PIH, PE, PP, PA, GDM, pPROM, PTB, SB, CD, SGA, LGA, NICU
Jacques et al. [14]	Retrospective case–	Nantes University	113	113	Endometriosis surgicall	I: 27(20.9 %)	64 (56.6)	65 (57.5)	$p > 0.05$	First trimester bleeding,

control study	Hospital, France	y diagnosed in 101 (78.3%) patients. For the other patients diagnosis via clinical examination and MRI	II: 36 (27.9%) III: 26 (20.2%) IV: 35 (27.1%) Ovarian: 77 (59.7%) DIE: 56 (43.4%) Peritoneal: 53 (41.1%) Adenomyosis: 12 (9.3%)	PE, PP, PTD, pPROM, IUGR, CD, GDM, GC, pelvic pains, PPH

Table 1. Characteristics of the included studies
APH antepartum hemorrhage, ART assisted reproductive technology, n/a not available, ASRM American Society for Reproductive Medicine, PIH pregnancy-induced hypertension, PE pre- eclampsia, PPH primary postpartum hemorrhage, PP placenta previa, PA placental abruption, pPROM preterm premature rupture of membranes, PTD preterm delivery, PPH postpartum hemorrhage, IUGR intra-uterine growth restriction, SGA small for gestational age, CD Cesarean delivery, GC gestational cholestasis, SB spontaneous birth, NICU neonatal intensive care unit admission, GDM gestational diabetes mellitus
[a]Data not presented separately in the two groups
[b]The control group in this study was women with spontaneous singleton pregnancies in the general population during 1996–2007 at the University Hospital of Kuopio. However, this could have included women with endometriosis. That is why for the current meta-analysis only women with pregnancy after ART and infertility reason other than endometriosis were considered as control group

In the comparison of women with and without endometriosis having undergone ART, the analysis of pooled data showed a significantly higher incidence in PP events in patients with endometriosis (OR 2.96 (95% CI 1.25–7.03); p = 0.01, I^2 = 69%, random-effect model) (Fig. 2).

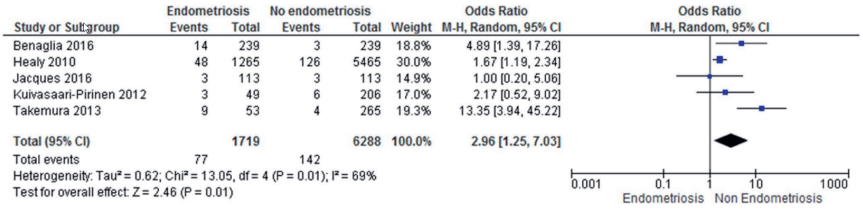

Figure 2. Placenta previa in women with and without endometriosis after ART

Placental abruption (PA)

In the comparison of women with and without endometriosis having undergone ART, the pooled analysis data showed no differences in PA events between the two groups (OR 0.44 (95% CI 0.10–1.87); p = 0.26, I^2 = 0%, fixed-effect model) (Fig. 3).

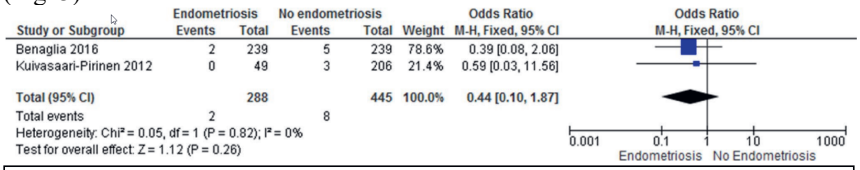

Figure 3. Placental abruption in women with and without endometriosis after ART

Discussion

In this meta-analysis evaluating pregnancy outcomes following ART, we found that the risk of PP after ART in endometriosis patients was threefold higher than those without endometriosis and this difference was statistically significant. No difference was found in the incidence of PA between the two groups. ART itself has been associated with an increased risk of pregnancy complications in non-randomized studies and found to be associated with a sixfold increased risk of PP [28-30]. The American College of Obstetricians and Gynecologists (ACOG) recently released recommendations on the management of these risks [31]. The present meta-analysis highlights that this risk is greater still when ART is performed in patients with endometriosis, suggesting that endometriosis is an additional and potentially independent risk factor of PP.

A recent meta-analysis examined the influence of endometriosis on the ART outcome of live births [32]. This study found a lower pregnancy rate among women with severe endometriosis. More recently, in women with endometriosis, the preterm birth risk was significantly increased in both spontaneous conception (OR 1.59, 95% CI 1.32–1.90) and ART (OR 1.43, 95% CI 1.14–1.79) [33]. Both the meta-analyses did not investigate the impact of endometriosis after ART on PP.

Known risk factors for PP are previous cesarean deliveries, maternal age, multiple pregnancy, multiparity, smoking, drug use and previous termination of pregnancy [34]. The mechanisms accounting for a higher risk of PP in women with endometriosis are largely unknown. This may be due to anomalous blastocyst implantation in the lower segment due to dysperistalsis and abnormal frequency and amplitude of uterine contractions observed in women with endometriosis [35]. Another explanation proposed previously is pelvic adhesions, secondary to peritoneal endometriosis, which may cause a fixed uterus leading to abnormal placental implantation. Placenta previa can also be a consequence of the profound structural and functional alterations observed in the endomyometrium of women with endometriosis. Some already described differences in the endometrium of women with endometriosis include lower peak endometrial thickness, progesterone resistance, altered local estrogen production and oxidative stress response as well as differences in cytokines, inflammatory mediators and apoptotic markers [36-39]. It has been shown

that various hormone therapies, such as progestins and GnRHa, may reduce cytokine concentration and inflammation in patients with endometriosis, thereby also suppressing the pathogenesis of the disease [40-42]. It remains to be determined whether such therapy used prior to ART is also influential in reducing the risk of PP in ART pregnancies. It is also unclear whether these patients would benefit from surgical pretreatment. Studies examining possible correlations between PP and the stage and type of endometriosis may also help to clarify possible links. Certainly, without these data, the performance of any form of prophylactic medical or surgical treatment to reduce the risk of PP would not be justified. The current study only demonstrates association and not causality between endometriosis and PP. Given the paucity of data currently available, a definitive evidence-based strategy for the management of endometriosis before ART cannot be determined.

In the general population of women with endometriosis with or without ART, the data considering PA are variable and do not allow clear conclusions to be drawn [10,15,17,20]. Moreover, there do not appear to be differences between patients undergoing ART with and without endometriosis.

The association between endometriosis and other placental diseases, such as placenta accreta could not be examined due to a paucity of existing data. However, an association would not be surprising since placenta accreta is known to be correlated with PP [43].

The limitations of this meta-analysis also require consideration. First, the relatively high heterogeneity in the PP out- come, which is related to the design of the studies included and by the magnitude and direction of effects. Unfortunately, subgroup analysis was not possible to investigate the impact of stage and type of endometriosis or previous cesarean deliveries because the details were not always provided. However, because systematic reviews bring together stud- ies that are diverse both clinically and methodologically, a certain degree of heterogeneity is expected [44]. Indeed, the distribution of observed values of I^2 derived from 509 meta-analyses in the Cochrane Database of Systematic Reviews revealed that about a quarter of the meta-analyses have I^2 values over 50% [45]. Furthermore, biases that may impact on the final set of included studies may also include publica- tion bias given the tendency to submit or accept manuscripts for publication based on the direction or strength of the study findings [46].

There are also inherent limitations in drawing conclusions from retrospective studies. However, the lack of prospec- tive trials comparing pregnancy outcome between women with and without endometriosis means that meta-analysis of retrospective studies remains the best available level of evidence.

The selection of the controls in the studies we assessed did not always require the exclusion of a diagnosis of endometriosis based on histology/surgery. However, this possible bias may be of limited relevance since the indication to ART in most of the patients in the control groups was male infertility. Nevertheless, the inclusion of even a small proportion of patients with endometriosis in the control groups would represent a selection bias and affect the results concerning the level of differentiation between the two groups. Limitations also include lack of information regarding the method of diagnosis in one study [28].

The lack of detailed data on previous cesarean deliveries should also be noted. Due to the well-established correlation between previous cesarean delivery and PP, a higher number of previous cesarean deliveries, and not endometriosis, may be the reason for a higher incidence of PP among endometriosis patients. However, the case and control groups included a similar number of nulliparas in most of the included studies; it is, therefore, unlikely that a history of a previous cesarean section may have significantly biased our results. Of note, Takemura et al. reported no PP after a history of previous cesarean delivery [23].

Many of the limitations outlined above are intrinsic limi- tations to controlled observation studies, and as previously stated, this remains the only available evidence to conduct this analysis.

In conclusion, the present meta-analysis suggests that endometriosis is associated with an increased risk of PP in pregnancies resulting after ART. Despite the inability to determine if endometriosis alone or endometriosis plus ART results in placental outcomes, physicians should be aware of the potential additional risk that endometriosis patients undergoing ART harbor. Patients with endometriosis undergoing ART should be counseled accordingly.

References:

1. Giudice LC, Kao LC (2004) Endometriosis. *Lancet* 364:1789–1799

2. Eskenazi B, Warner ML (1997) Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 24:235–258

3. Qin JB, Sheng XQ, Wang H et al (2017) Worldwide prevalence of adverse pregnancy outcomes associated with in vitro fertilization/intracytoplasmic sperm injection among multiple births: a systematic review and meta-analysis based on cohort studies. *Arch Gynecol Obstet* 295:577–597.

4. Karami M, Jenabi E, Fereidooni B et al (2017) The association of placenta previa and assisted reproductive techniques: a meta- analysis. *J Matern Fetal Neonatal Med* 6:1–8

5. McArthur JW, Ulfelder H (1965) The effect of pregnancy upon endometriosis. *Obstet Gynecol Surv* 20:709–733

6. Aris A (2014) A 12-year cohort study on adverse pregnancy outcomes in Eastern Townships of Canada: impact of endometriosis. *Gynecol Endocrinol* 30:34–37

7. Benaglia L, Candotti G, Papaleo E et al (2016) Pregnancy out- come in women with endometriosis achieving pregnancy with IVF. *Hum Reprod* 31:2730–2736

8. Brosens IA, De Sutter P, Hamerlynck T et al (2007) Endometri- osis is associated with a decreased risk of pre-eclampsia. *Hum Reprod* 22:1725–1729

9. Conti N, Cevenini G, Vannuccini S et al (2015) Women with endometriosis at first pregnancy have an increased risk of adverse obstetric outcome. *J Matern Fetal Neonatal Med* 28:1795–1798

10. Exacoustos C, Lauriola I, Lazzeri L et al (2016) Complications during pregnancy and delivery in women with untreated rec- tovaginal deep infiltrating endometriosis. *Fertil Steril* 106:1129

11. Fernando S, Breheny S, Jaques AM et al (2009) Preterm birth, ovarian endometriomata, and assisted reproduction technologies. *Fertil Steril* 91:325–330

12. Glavind MT, Forman A, Arendt LH et al (2017) Endometrio- sis and pregnancy complications: a Danish cohort study. *Fertil Steril* 107:160–166

13. Hadfield RM, Lain SJ, Raynes-Greenow CH et al (2009) Is there an association between endometriosis and the risk of pre-eclampsia? A population based study. *Hum Reprod* 24:2348–2352

14. Jacques M, Freour T, Barriere P et al (2016) Adverse pregnancy and neo-natal outcomes after assisted reproductive treatment in patients with pelvic endometriosis: a case–control study. *Reprod Biomed Online* 32:626–634

15. Kortelahti M, Anttila MA, Hippelainen MI et al (2003) Obstetric outcome in women with endometriosis–a matched case–control study. *Gynecol Obstet Investig* 56:207–212

16. Kuivasaari-Pirinen P, Raatikainen K, Hippelainen M et al (2012) Adverse outcomes of IVF/ICSI pregnancies vary depending on aetiology of infertility. ISRN *Obstet Gynecol* 2012:451915

17. Lin H, Leng JH, Liu JT et al (2015) Obstetric outcomes in Chinese women with endometriosis: a retrospective cohort study. *Chin Med J (Engl)* 128:455–458

18. Mannini L, Sorbi F, Noci I, Ghizzoni V et al (2017) New adverse obstetrics outcomes associated with endometriosis: a retrospective cohort study. *Arch Gynecol Obstet* 295:141–151

19. Mekaru K, Masamoto H, Sugiyama H et al (2014) Endometriosis and pregnancy outcome: are pregnancies complicated by endo- metriosis a high-risk group? *Eur J Obstet Gynecol Reprod Biol* 172:36–39

20. Saraswat L, Ayansina DT, Cooper KG et al (2017) Pregnancy outcomes in women with endometriosis: a national record linkage study. *BJOG* 124:444–452

21. Stephansson O, Kieler H, Granath F et al (2009) Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Hum Reprod* 24:2341–2347

22. Stern JE, Luke B, Tobias M et al (2015) Adverse pregnancy and birth outcomes associated with underlying diagnosis with and without assisted reproductive technology treatment. *Fertil Steril* 103:1438–1445

23. Takemura Y, Osuga Y, Fujimoto A et al (2013) Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. *Gynecol Endocrinol* 29:113–115

24. Maggiore ULR, Ferrero S, Mangili G et al (2016) A systematic review on endometriosis during pregnancy: diagnosis, misdiagno- sis, complications and outcomes. *Hum Reprod Update* 22:70–103

25. Leone Roberti Maggiore U, Inversetti A, Schimberni M et al (2017) Obstetrical complications of endometriosis, particularly deep endometriosis. *Fertil Steril* 108:895–912

26. Zullo F, Spagnolo E, Saccone G et al (2017) Endometriosis and obstetrics complications: a systematic review and meta-analysis. *Fertil Steril* 108:667–672

27. Bulun SE (2009) Mechanisms of disease endometriosis. *N Engl J Med* 360:268–279

28. Healy DL, Breheny S, Halliday J et al (2010) Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. *Hum Reprod* 25:265–274

29. Romundstad LB, Romundstad PR, Sunde A et al (2006) Increased risk of placenta previa in pregnancies following IVF/ICSI; a com- parison of ART and non-ART pregnancies in the same mother. *Hum Reprod* 21:2353–2358

30. Shevell T, Malone FD, Vidaver J et al (2005) Assisted repro- ductive technology and pregnancy outcome. *Obstet Gynecol* 106:1039–1045

31. Sumners J, Ecker JL, Practice CO et al (2016) Perinatal risks associated with assisted reproductive technology. *Obstet Gynecol* 128:E61–E68

32. Hamdan M, Omar SZ, Dunselman G et al (2015) Influence of endometriosis on assisted reproductive technology outcomes a systematic review and meta-analysis. *Obstet Gynecol* 125:79–88

33. Pérez-López FR, Villagrasa-Boli P, Muñoz-Olarte M et al (2018) Association between endometriosis and preterm birth in women with spontaneous conception or using assisted reproductive tech- nology: a systematic review and meta-analysis of cohort studies. *Reprod Sci* 25:311–319

34. Oyelese Y, Smulian JC (2006) Placenta previa, placenta accreta, and vasa previa. *Obstet Gynecol* 107:927–941

35. Bulletti C, De Ziegler D, Polli V et al (2002) Characteristics of uterine contractility during menses in women with mild to moder- ate endometriosis. *Fertil Steril* 77:1156–1161

36. Aghajanova L, Tatsumi K, Horcajadas JA et al (2011) Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. *Biol Reprod* 84:801–815

37. Benagiano G, Bastianelli C, Farris M et al (2014) Selective pro- gesterone receptor modulators: an update. *Expert Opin Pharmacother* 15:1403–1415

38. Bromer JG, Aldad TS, Taylor HS (2009) Defining the proliferative phase endometrial defect. *Fertil Steril* 91:698–704

39. Burney RO, Talbi S, Hamilton AE et al (2007) Gene expression analysis of endometrium reveals progesterone resistance and can- didate susceptibility genes in women with endometriosis. *Endocrinology* 148:3814–3826

40. Grandi G, Mueller M, Bersinger N et al (2016) Progestin sup- pressed inflammation and cell viability of tumor necrosis factor- alpha-stimulated endometriotic stromal cells. *Am J Reprod Immu- nol* 76:292–298

41. Nirgianakis K, Bersinger NA, McKinnon B et al (2013) Regres- sion of the inflammatory microenvironment of the peritoneal cavity in women with endometriosis by GnRHa treatment. *Eur J Obstet Gynecol Reprod Biol* 170:550–554

42. Nirgianakis K, Grandi G, McKinnon B et al (2016) Dienogest mediates midkine suppression in endometriosis. *Hum Reprod* 31:1981–1986

43. Miller DA, Chollet JA, Goodwin TM (1997) Clinical risk fac- tors for placenta previa-placenta accreta. *Am J Obstet Gynecol* 177:210–214

44. Higgins J, Thompson S, Deeks J et al (2002) Statistical heteroge- neity in systematic reviews of clinical trials: a critical appraisal of guidelines and practice. *J Health Serv Res Policy* 7:51–61

45. Higgins JPT, Thompson SG, Deeks J (2003) Measuring inconsist- ency in meta-analyses. *BMJ* 327:557–560

46. Song F, Eastwood AJ, Gilbody S et al (2000) Publication and related biases. Health Technol Assess 4:1–1151. Barnea ER. Insight into early pregnancy events: The emerging role of the embryo. *Am J Reprod Immunol* 2004 51(5):319–322.

Chapter 6

Obstetric complications after laparoscopic excision of posterior deep infiltrating endometriosis: a case–control study

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Abstract

Objective: To study obstetric outcomes and complications in women with previously excised posterior deep infiltrating endometriosis (DIE) in comparison with women without endometriosis.

Design: Matched case–control study.

Setting: Tertiary-level academic center.

Patient(s): All surgeries for endometriosis performed in the Department of Gynecology and Gynecological Oncology, University of Bern between March 2004 and July 2015, were assessed. Inclusion criteria included complete laparoscopic excision of posterior DIE. Exclusion criteria included concomitant hysterectomies, refusal to participate, and patients lost to follow-up. Each subsequent pregnancy was matched to three controls by maternal age, parity, history of cesarean, and mode of conception.

Intervention(s): None.

Main Outcome Measure(s): Obstetric complications.

Result(s): Among 841 patients with surgically diagnosed endometriosis, 125 satisfied the inclusion and exclusion criteria. Of these, 73 pregnancies resulted, although a further 11 patients were excluded owing to early miscarriages or extrauterine pregnancies. The final study cohort included 62 singleton pregnancies matched to 186 controls. The analysis identified an increased risk of placenta previa, gestational hypertension, and intrauterine growth restriction for the case group. The possibility of successful vaginal delivery was similar between groups. Moreover, no significant increase in risk of maternal and neonatal delivery complications, except for a slightly higher postpartum blood loss in the case group, was observed.

Conclusion(s): Despite previous surgical excision, women with history of DIE present a higher risk of placenta previa, gestational hypertonia, and intrauterine growth restriction during pregnancy. Previous surgery for DIE does not seem to predispose to failed vaginal delivery.

Introduction

Endometriosis is an extremely heterogeneous disease broadly separated into three distinct cat- egories: superficial peritoneal, ovarian, and deep infiltrating endometriosis (DIE). Deep infiltrating endometriosis lesions are characterized by penetration in excess of 5 mm under the peritoneal surface [1]. They are found in many locations, most commonly in the rectouterine pouch [2], and can involve uterosacral ligaments, the posterior vaginal wall, the anterior rectal wall, and in most severe cases, extend laterally with ureteral involvement [3]. Symptoms may include dyschezia, bowel dysfunction, dyspareunia, and lower abdominal pain. Surgical excision is a common treatment option for symptomatic cases because it reduces pain and improves quality of life [4].

Over the past few years it has emerged that endometriosis may impact pregnancy outcomes. A series of controlled observational studies have shown a negative association with endometriosis [5-14] that was confirmed in systematic meta-analysis [15,16]. However, most of these studies do not focus on DIE; nor do they provide surgical treatment information. This is crucial because pregnancy complications may differ according to the endometriotic lesion [17] or mode of surgery. Moreover, these studies have mainly examined pregnancy but not critical delivery outcomes, such as the rate of failed vaginal delivery or severe birth trauma. As a result, the proper delivery management of these patients remains unclear. One recent study found increased risk of obstetric complications in women with untreated posterior DIE [18]. It has not yet been examined whether a similar risk persists after complete excision of DIE [19].

Complete surgical removal of symptomatic posterior DIE with or without vaginal and bowel involvement is regularly performed in specialized centers, with many women achieving pregnancy after surgery [20]. It is of major importance therefore to identify potential pregnancy and delivery complications and establish evidence-based management policies in this specific group of patients. In the present study we examined the effect of a complete laparoscopic excision of posterior DIE on subsequent pregnancy and delivery outcomes.

Materials and methods

The study was prepared according to the ‘‘Strengthening the reporting of observational studies in epidemiology’’ guidelines [21] and was institution review board approved (no. 2016-00402).

In this matched case–control study, the case group was derived from all patients with laparoscopically treated poste- rior DIE in the Department of Gynecology and Gynecological Oncology, University of Bern, between March 2004 and July 2015. Only women with complete excision of posterior DIE, histologically verified, were included in the study. The following outcomes potentially related to pregnancy and de- livery risks, were collected: [1] type of bowel surgery (shaving, segmental, or disc bowel resection), [2] revised American Society for Reproductive Medicine (rASRM) stage, [3] affected structures, [4] level of bowel anastomosis and length of resected bowel, if performed, [5] partial resection of posterior vaginal fornix, [6] concomitant bladder wall resection, and [7] protective stoma. All women were contacted via post, and a written informed consent form, as well as a completed questionnaire on pregnancies and delivery outcomes, was obtained. Multiple pregnancies and pregnancies before the surgery were excluded. The detailed

outcomes (parity, time between endometriosis surgery and conception, mode of conception, duration of pregnancy, pregnancy and delivery complications, mode of delivery, newborn birth weight, Apgar score, and umbilical blood gases) were obtained from the obstetric clinics where medical care was provided.

The control group was obtained from all women with early pregnancy (12–15 weeks of pregnancy) presenting to the Ultrasound Department of Obstetrics and Gynecology, University of Bern from March 2014 to November 2016. Their pregnancy and delivery outcomes are stored and recorded in a newborn registration database of the Ultrasound Department. Women with documented endometriosis or adenomyosis were excluded. The case and control groups were matched for age, parity, previous cesarean section, and mode of conception. Three control pregnancies were matched to each case pregnancy.

Surgical Technique

The standardized laparoscopic surgical technique performed in our clinic has been described previously [22]. Briefly, the rectovaginal septum is dissected and the nodule mobilized. Vaginal infiltration is treated by partial resection of the posterior vaginal fornix. All lesions are initially treated by shaving alone. When necessary, deeply infiltrative rectal lesions are treated by either segmental or disc resection, depending on the circumference and length of rectal involvement. In cases with extensive involvement and when disc resection is deemed inadequate for macroscopic clearance, segmental resection is performed. Defunctioning ileostomies are performed selectively. The removal of all endometriotic implants is pursued.

Definitions

Gestational complications were defined as follows: preterm birth was delivery before 37 completed weeks of gestation; gestational hypertension was blood pressure persistently over 140/90 mm Hg developed after 20 weeks of gestation in a previously normotensive woman; pre-eclampsia was gestational hypertension and proteinuria (>300 mg/24 hours); gestational diabetes was a carbohydrate intolerance with onset in pregnancy with a positive oral glucose tolerance test; small for gestational age (SGA) was an infant weighing less than the 10th percentile according to the fetal growth curve; intrauterine growth restriction (IUGR) indicated an infant weighing less than the 3rd percentile or less than the 10th with pathologic Doppler cerebro-placental ratio, umbilical ar- tery or uterine arteries flows; placental abruption was separa- tion of the placenta from its site of implantation before delivery; and placenta previa was complete or partially covering of the internal cervical os during the third trimester. Postpartum hemorrhage (PPH) was defined as the loss of more than 500 mL or 1000 mL blood within the first 24 hours after childbirth after vaginal or cesarean delivery, respectively.

Statistical Analysis

Descriptive statistical and binary logistic regression analyses were performed. A Student t test and Mann–Whitney U test were used to compare continuous parametric and nonpara- metric variables, respectively. Fisher's exact test was used to compare binary variables. Univariate and multivariate anal- ysis were performed to analyze factors predicting unfavorable pregnancy or delivery outcomes. The variables included in the model showed a Wald test's parameter different from 0. If

the Wald test showed that the parameter for a variable was zero, the variable was removed from the model. Multivariate models were performed for variables with a P value of ≤ .3 in the univariate analysis. P values of ≤ .05 were considered statistically significant. Statistical analysis was carried out with GraphPad Prism version 6.0 (GraphPad Software) and IBM-Microsoft SPSS version 22.0.

Results

During the study period, among 841 patients with laparoscopically diagnosed endometriosis, 222 patients underwent a complete excision of posterior DIE. Forty-nine were lost to follow-up, 12 refused to participate in the study, and 36 were excluded owing to concomitant hysterectomy. From the remaining 125 women in the study, 73 pregnancies were documented. Ten pregnancies (13.7%) resulted in miscarriages in the first trimester of pregnancy, and one (1.4%) in an extrauterine pregnancy. Because the control group included only pregnancies after first trimester ultrasound screening, neither early miscarriages nor extrauterine pregnancies were expected. Consequently, the 10 miscarriages and one extrauterine pregnancy were excluded from the final case group, resulting in a final study cohort of 62 singleton pregnancies (Fig. 1). The control group consisted of 186 pregnancies.

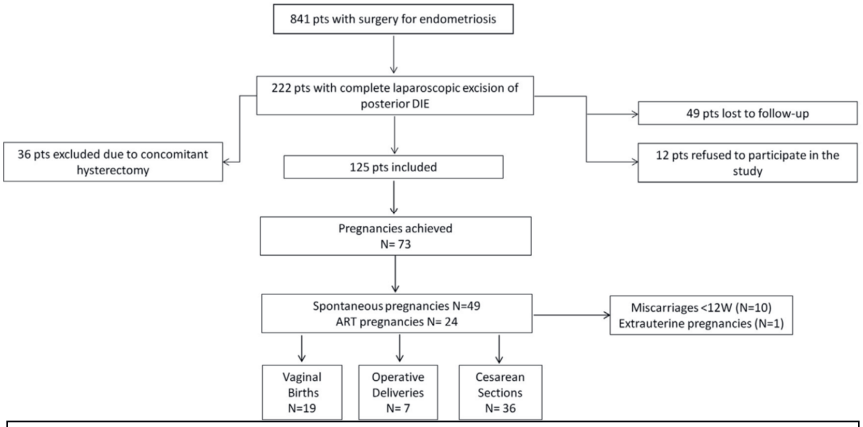

Figure 1. Flow diagram of the case group. ART = assisted reproductive technology; pts= patients

The baseline characteristics of the groups, including, time from surgery to conception, endometriosis rASRM stage, and surgical outcomes, are presented in Table 1. In one woman, a protective ileostomy was performed owing to ultralow bowel anastomosis (4 cm ab ano). Cases and controls had no statistically significant differences in terms of age, body mass index, parity, previous uterine surgery, and type of conception (Table 1).

Characteristic	Endometriosis (n = 62)	Controls (n = 186)	*P* value
Age at delivery (y)	33.7 ± 3.74	33.8 ± 4.38	ns
BMI (kg/m^2)	23.5 ± 3.77	22.9 ± 3.57	ns
Previous deliveries			ns

None	40 (64.5)	125 (67.2)	
1	20 (32.3)	55 (29.6)	
2	2 (3.2)	6 (3.2)	
Previous cesarean section	9 (14.5)	26 (14)	ns
Mode of conception			ns
Spontaneous	40 (64.5)	123 (66.1)	
Insemination or/and hormonal stimulation	7 (11.3)	12 (6.5)	
IVF/ICSI	15 (24.2)	51 (27.4)	
Time from surgery to conception (mo)	25 (1–110)	–	–
Segmental bowel resection	29 (46.5)	–	–
Disc resection	4 (6.5)	–	–
Bowel shaving	25 (40.3)	–	–
Length of removed bowel (cm)	7 (3.5–13)	–	–
Distance of bowel anastomosis ab ano (cm)	8 (4–30)	–	–
Partial resection of posterior vaginal fornix	37 (60)	–	–
Concomitant bladder wall resection	3 (4.8)	–	–
Protective stoma	1 (1.6)	–	–

Table 1. Baseline characteristics of the two groups.
Note: Data presented as mean ± standard deviation or median (range) for continuous variables and number (percentage) for the qualitative variables. Comparison between groups using the Student t test and Fisher's exact test as appropriate. ICSI = intracytoplasmic sperm injection; ns = non significant

Pregnancies after surgery for posterior DIE showed a higher risk of placenta previa (P= .004), gestational hypertension (P= .036), and IUGR (P= .0496). The incidence of vaginal delivery was lower in the DIE group; however, this wasmarginal and not statistically significant (P= .056). Indeed, 26 of the 42 women who attempted vaginal delivery in the DIE group (61.9%) were successful. One delivery (2.4%) was complicated by a fourth-degree tear after vacuum delivery and another (2.4%) by a third-degree tear after spontaneous birth, similar to the control group. Ten deliveries (16.1 %) were accompanied by PPH in the DIE group; five after cesarean section and five after vaginal delivery, with blood transfusion necessary in five (8.1%). The risk of PPH was, however, not statistically significant different between groups (P .099). Blood loss was, conversely, significantly higher in the DIE group (P .006). Finally, no difference was observed in fetal acidosis or asphyxia between groups (Table 2).

Pregnancy and delivery outcomes	Endometriosis (n=62)	Controls (n=186)	*P* value	RR (95% CI)
Premature delivery <37 wk	8 (12.9)	13 (7)	ns	1.817 (0.79–4.18)
Premature delivery <32 wk	1 (1.6)	2 (1.1)	ns	1.5 (0.138–16.27)
Placenta previa	4 (6.5)	0	.004	n/a
Placental abruption	1 (1.6)	0	ns	n/a
Gestational diabetes	7 (11.3)	14 (7.5)	ns	1.5 (0.634–3.547)
Pre-eclampsia	3 (4.8)	5 (2.7)	ns	1.8 (0.443–7.318)
Gestational hypertension	4 (6.5)	2 (1.1)	.036	6 (1.126–31.98)
SGA	7 (11.3)	13 (7)	ns	1.615 (0.675–3.867)
IUGR	7 (11.3)	7 (3.8)	.0496	3 (1.095–8.218)
Spontaneous vaginal delivery	19 (30.7)	66 (35.5)	ns	0.864 (0.566–1.32)
Instrumental vaginal delivery	7 (11.3)	39 (21)	ns	0.538 (0.254–1.14)
Primary cesarean section	20 (32.3)	39 (21)	.085	1.538 (0.975–2.428)
Secondary cesarean section	16 (25.8)	42 (22.6)	ns	1.111 (0.583–2.117)

Normal vaginal delivery (spontaneous and instrumental)	26 (41.9)	105 (56.5)	.0564	0.743 (0.54–1.022)
Failed vaginal delivery	16 (38.1[a])	42 (28.6[b])	ns	1.33 (0.839-2.12
Second-degree perineal tear or episiotomy	11 (26.2[a])	68 (36.6[b])	ns	0.716 (0.417-1.23
Third- or fourth-degree perineal or button hole tear	2 (4.8[a])	5 (3.4[b])	ns	1.4 (0.2815–6.962)
Vaginal laceration	3 (7.1[a])	14 (9.5[b])	ns	0.75 (0.226–2.49)
PPH	10 (16.1)	16 (8.6)	.0995	1.875 (0.898–3.915)
Blood loss (mL)	500 (200–2000)	400 (200–1500)	.0063	
phA < 7.10	1 (1.6)	6 (3.2)	ns	0.505 (0.062–4.12)
phA	7.28 (7.05–7.38)	7.26 (6.92–7.42)	ns	
phV	7.35 (7.15–7.47)	7.35 (7.02–7.49)	ns	
5-minute Apgar score	9 (1–10)	9 (1–10)	ns	

Table 2. Pregnancy and delivery outcomes between groups.
Note: Data presented as mean ± standard deviation or median (range) for continuous variables and number (percentage) for the qualitative variables. Comparison between groups using the Student t test, Mann–Whitney U test, and Fisher's exact test as appropriate. CI= confidence interval; RR= relative risk.
[a] Percentage of patients attempting a vaginal delivery (n= 42) in the case group
[b] Percentage of patients attempting a vaginal delivery (n= 147) in the control group

Out of 26 women with successful vaginal delivery, 14 (53.8%) had previous partial vaginal fornix resection, 14 (53.8%) previous bowel segment resection, 4 (15.4%) bowel disc resection, and 8 (30.8%) bowel shaving. Five of these de- liveries (19.2%) were complicated with PPH; however, all of them were due to placenta retention or atonia and not due to birth trauma. No case of severe laceration of the upper vagina was reported in any patient in both groups.

The indications for either primary or secondary cesarean section in the endometriosis group are presented in Table 3. One of these indications was directly correlated with DIE (intra-abdominal bleeding with hematoperitoneum due to endometriosis lesion). In one woman with previous cesarean section and uterine contractions at 38 weeks a repeat cesarean section was performed, revealing a uterine perforation. In another with placenta previa a cesarean section with concomitant supracervical hysterectomy was performed because of associated placenta accreta.

Indication for cesarean section	No. of cesarean sections (Total=36)
Primary cesarean sections	20
Breech presentation	6
Previous cesarean section	6
Placenta previa	4
Previous traumatic vaginal birth	1
Intra-abdominal bleeding due to endometriosis	1
Perianal thrombosis in the 31st week of pregnancy	1
Pre-eclampsia	1
Secondary cesarean sections	16
Labor dystocia	6
Pathological cardiotocography	6
Amniotic infection syndrome	2
Extensive vaginal bleeding during labor	1
Nondefined	1

Table 3. Indication for cesarean section in the endometriosis group

The univariate and multivariate analysis of possible risk factors for cesarean delivery showed that bowel anastomosis during DIE surgery was positively associated with cesarean delivery (P= .04). None of the examined factors was associated with PPH.

Discussion

In the present study we demonstrate that women with excised posterior DIE, similarly to women with endometriosis in general, have a statistically significant increased risk of placenta previa, gestational hypertension, and IUGR compared with women without endometriosis. An important finding of the study was that the possibility of successful vaginal birth, if attempted, was high and similar to that in the control group. Except for a higher postpartal blood loss in the endometriosis group, all other delivery and neonatal risks were similar between groups. History of bowel anastomosis during DIE surgery was positively associated with delivery via cesarean section.

To the best of our knowledge this is the first controlled study to assess the pregnancy and delivery outcomes in patients who have previously undergone complete laparoscopic excision of posterior DIE. Recently an increased risk of preterm birth, placenta previa, gestational hypertension, and cesarean section in women with posterior nontreated DIE has been identified [17]. However, because of the unmatched design of this study, significant differences in terms of age, previous uterine surgery, parity, and mode of conception between groups may have biased the results. More specifically, almost half of pregnancies were achieved after assisted reproductive technology in the endometriosis group, compared with none in the control group. Because assisted reproductive technology is associated per se with a higher risk of cesarean section and other pregnancy complications [23], this may have biased the results significantly. Nevertheless, our results partially support those of the earlier study, which associate DIE with pregnancy complications, and extend them to show that surgical removal of DIE at least does not seem to increase obstetric risks.

Previous studies report different influences of endometriosis on pregnancy [5-14, 24-30]. A recent meta-analysis concluded there was a higher risk of preterm birth, placenta previa, SGA, and cesarean delivery [15], similar to what we have observed. However, the classification of endometriosis and the surgery performed was poorly documented in most of these studies, and the case groups consisted of any type of endometriosis, mainly peritoneal and ovarian, thus signif- icantly differing from our study population.

Our study suggests that complete excision of posterior DIE does not prevent the risk of placenta previa often reported in women with endometriosis. Patients should thus be informed that this type of surgery will not reduce the probability of this pregnancy complication and the risks associated with it. There are several theories linking endometriosis and placenta previa, including dysperistalsis and abnormal uterine contractions in women with endometriosis leading to anomalous blastocyst implantation [31]. Pelvic adhesions secondary to endometriosis and causing a fixed uterus may also contribute, as could an aberrant, inflammatory intrauterine environment. The association with gestational hypertension and IUGR could be

related to the thickening of the junctional zone reported in endometriosis [32-34], because trophoblastic invasion into this layer is critical for pregnancy [35]. Abnormal spiral artery remodeling, inflammation, oxidative stress, and an imbalance in the angiogenic milieu of the endometrium may also be reasons for abnormal placentation [36]. Consequently, previous surgery for DIE is highly unlikely to represent the cure for these abnormalities because these represent mainly a pre-existing predisposition rather than a consequence of DIE lesion presence.

In contrast to the study of Exacoustos et al. [18], which identified a very high incidence of preterm birth in women with nontreated posterior DIE (31.7%), no similar risk was observed in women with surgically treated posterior DIE in our study. Whether this was solely due to the surgical excision of endometriosis cannot be answered with certainty but represents an intriguing possibility.

It is reasonable to assume that the higher risk of placenta previa, gestational hypertension, and IUGR associated with DIE would lead to a higher incidence of cesarean delivery. In contrast to previous studies, though, we found a higher, but nonsignificant (P= .0564) increase in the incidence of cesarean delivery in the case group. This may be due to the specific subgroup of women analyzed in this study or a positive influence of surgical removal of DIE on subsequent vaginal deliveries. However, given the borderline nature of the results, this may be simply statistical variation. Nevertheless, when a woman in the DIE group without contraindication attempted a vaginal delivery, the success rate was similar to that in the control group (61.9% vs. 71.4%), meaning previous surgery for posterior DIE does not predispose to vaginal delivery failure.

A previous study reported a significant influence of vaginal involvement and bowel resection on the method of delivery [37]. Our findings also associate bowel anastomosis but not partial resection of the posterior vaginal fornix due to DIE with an increased incidence of cesarean delivery in subsequent pregnancies. It is likely that this association originates from a biased choice toward cesarean deliveries by clinicians due to concerns about possible obstetric complications after such surgeries. Nevertheless, because of the wide confidence interval in the multivariate analysis, this finding should be interpreted with caution.

Previous partial resection of the posterior vaginal fornix and the resulting scar could lead to severe lacerations of the upper vagina during labor, which represents a potentially major complication, in terms of blood loss and difficulties at su- turing. However, no such complication has been reported in any patient in our study. Similarly, in the study by Allerstorfer et al. [37] no vaginal laceration was documented after previous excision of vaginal endometriosis. Given such a low incidence of this complication, our study is underpowered to detect a between-group risk difference. Because of its potential severity, large multicenter studies are needed to better elucidate its incidence and clinical significance. In the meantime it should be carefully considered by all obstetricians caring for pregnant women with previous surgery for posterior DIE.

The higher postpartum blood loss but not higher rate of PPH observed in the endometriosis group could be explained by the increased incidence of cesarean delivery in this group, known to have higher blood loss (58.1% vs. 43.6%). Conse-

quently, it cannot be concluded that previous surgery for posterior DIE is responsible per se for higher postpartum blood loss.

One limitation of our study is that low sample numbers limit the incidences of some rarer events. For example, despite the high number of pregnancies in the control group, none presented with placenta previa or placenta abruption. For the same reason the validity of the univariate and multivariate analysis for potential risk factors related to cesarean section and PPH is limited. Moreover, as stated above, many patients who underwent complete excision of posterior DIE were not included, because they were lost to follow-up or refused participation. Considering these patients are often at increased risk of unfavorable outcome, this constitutes a drawback of the study. The strengths of the study, however, include the clear inclusion criteria of patients only with completely excised severe posterior DIE, with high incidence of bowel surgery and partial resection of posterior vaginal fornix, as well as the comparison with a controlled group matched for the most important confounding outcomes, thus increasing the quality and enhancing the interpretation of the results.

In conclusion, whether a complete surgical treatment of DIE endometriosis has a beneficial influence on pregnancy and delivery cannot be directly answered from the present study. It does, however, suggest that no additional adverse effects not already related to the presence of endometriosis itself are created. The clinician should be aware of the potential risk of placenta previa, gestational hypertension, and IUGR associated with previous posterior DIE; however, a change in prenatal care cannot be suggested, because the standard prenatal care in developed countries should be adequate to diagnose these complications early enough in the pregnancy. Last, concerns that the surgery of the rectovaginal septum with or without bowel or vaginal involvement may predispose to failed vaginal delivery are refuted by this study. Women trying to deliver vaginally succeed with a similar rate as endometriosis-free women, although further studies with increased numbers of patients delivering vaginally are required to conclusively determine the safety of vaginal delivery in this specific group of patients.

References

1. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertil Steril* 1991, 55: 759–65.

2. Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V, et al. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. *Hum Reprod* 2003, 18:157–61.

3. Knabben L, Imboden S, Fellmann B, Nirgianakis K, Kuhn A, Mueller MD. Urinary tract endometriosis in patients with deep infiltrating endometriosis: prevalence, symptoms, management, and proposal for a new clinical classification. *Fertil Steril* 2015, 103: 147–52.

4. Meuleman C, Tomassetti C, Wolthuis A, Van Cleynenbreugel B, Laenen A, Penninckx F, et al. Clinical outcome after radical excision of moderate-severe endometriosis with or without bowel resection and reanastomosis: a pro- spective cohort study. *Ann Surg* 2014, 259: 522–31.

5. Takemura Y, Osuga Y, Fujimoto A, Oi N, Tsutsumi R, Koizumi M, et al. Increased risk of placenta previa is associated with endometriosis and tubal factor infertility in assisted reproductive technology pregnancy. *Gynecol Endocrinol* 2012, 29: 113–5.

6. Aris A. A 12-year cohort study on adverse pregnancy outcomes in Eastern Townships of Canada: impact of endometriosis. *Gynecol Endocrinol* 2013, 30: 34–7.

7. Conti N, Cevenini G, Vannuccini S, Orlandini C, Valensise H, Gervasi M, et al. Women with endometriosis at first pregnancy have an increased risk of adverse obstetric outcome. *J Matern Fetal Neonatal Med* 2014, 28: 1795–8.

8. Lin H, Leng JHH, Liu JTT, Lang JHH. Obstetric outcomes in Chinese women with endometriosis: a retrospective cohort study. *Chin Med J* 2015, 128: 455–8.

9. Stern J, Luke B, Tobias M, Gopal D, Hornstein M, Diop H. Adverse pregnancy and birth outcomes associated with underlying diagnosis with and without assisted reproductive technology treatment. *Fertil Steril* 2015, 103: 1438–45.

10. Benaglia L, Candotti G, Papaleo E, Pagliardini L, Leonardi M, Reschini M, et al. Pregnancy outcome in women with endometriosis achieving pregnancy with IVF. *Hum Reprod* 2016, 31: 2730–6.

11. Glavind MT, Forman A, Arendt LH, Nielsen K, Henriksen TB. Endometriosis and pregnancy complications: a Danish cohort study. *Fertil Steril* 2017, 107: 160–6.

12. Mannini L, Sorbi F, Noci I, Ghizzoni V, Perelli F, Tommaso M, et al. New adverse obstetrics outcomes associated with endometriosis: a retrospective cohort study. *Arch Gynecol Obstet* 2016, 295: 141–51.

13. Saraswat L, Ayansina D, Cooper K, Bhattacharya S, Miligkos D, Horne A, et al. Pregnancy outcomes in women with endometriosis: a national record linkage study. *BJOG* 2017, 124: 444–52.

14. Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. *Hum Reprod* 2009, 24: 2341–7.

15. Zullo F, Spagnolo E, Saccone G, Acunzo M, Xodo S, Ceccaroni M, et al. Endometriosis and obstetrics complications: a systematic review and meta- analysis. *Fertil Steril* 2017, 108: 667–72.e5.

16. Gasparri ML, Nirgianakis K, Taghavi K, Papadia A, Mueller MD. Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis. *Arch Gynecol Obstet* 2018, 298: 27–34.

17. Vercellini P, Parazzini F, Pietropaolo G, Cipriani S, Frattaruolo MP, Fedele L. Pregnancy outcome in women with peritoneal, ovarian and rectovaginal endometriosis: a retrospective cohort study. *BJOG* 2012, 119: 1538–43.

18. Exacoustos C, Lauriola I, Lazzeri L, De Felice G, Zupi E. Complications during pregnancy and delivery in women with untreated rectovaginal deep infiltrating endometriosis. *Fertil Steril* 2016,106: 1129–35.e1.

19. Leone Roberti Maggiore U, Inversetti A, Schimberni M, Vigano` P, Giorgione V, Candiani M. Obstetrical complications of endometriosis, particularly deep endometriosis. *Fertil Steril* 2017,108: 895–912.

20. Meuleman C, Tomassetti C, D'Hoore A, Cleynenbreugel B, Penninckx F, Vergote I, et al. Surgical treatment of deeply infiltrating endometriosis with colorectal involvement. *Hum Reprod Update* 2011, 17: 311–26.

21. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting obser- vational studies. *Lancet* 2007, 370: 1453–7.

22. Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller MD. Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence. *Acta Obstet Gynecol Scand* 2014, 93: 1262–7.

23. Qin JBB, Sheng XQQ, Wu D, Gao SYY, You YPP, Yang TBB, et al. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic re- view and meta-analysis. *Arch Gynecol Obstet* 2017, 295: 285–301.

24. Brosens I, Sutter P, Hamerlynck T, Imeraj L, Yao Z, Cloke B, et al. Endometri- osis is associated with a decreased risk of pre-eclampsia. *Hum Reprod* 2007, 22: 1725–9.

25. Hadfield R, Lain S, Raynes-Greenow C, Morris J, Roberts C. Is there an asso- ciation between endometriosis and the risk of pre-eclampsia? A population based study. *Hum Reprod* 2009, 24: 2348–52.

26. Kuivasaari-Pirinen P, Raatikainen K, Hippel€ainen M, Heinonen S. Adverse outcomes of IVF/ICSI pregnancies vary depending on aetiology of infertility. ISRN *Obstet Gynecol* 2012, 2012: 1–5.

27. Mekaru K, Masamoto H, Sugiyama H, Asato K, Heshiki C, Kinjyo T, et al. Endometriosis and pregnancy outcome: are pregnancies complicated by endometriosis a high-risk group? *Eur J Obstet Gynecol Reprod Biol* 2014, 172: 36–9.

28. Jacques M, Freour T, Barriere P, Ploteau S. Adverse pregnancy and neo- natal outcomes after assisted reproductive treatment in patients with pelvic endometriosis: a case–control study. *Reprod Biomed Online* 2016;32:626–34.

29. Kortelahti M, Anttila MA, Hippelainen MI, Heinonen ST. Obstetric outcome in women with endometriosis—a matched case-control study. *Gynecol Obstet Invest* 2003, 56: 207–12.

30. Chen I, Lalani S, Xie RHH, Shen M, Singh SS, Wen SWW. Association be- tween surgically diagnosed endometriosis and adverse pregnancy out- comes. *Fertil Steril* 2018;109:142–7.

31. Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A, Giorgione V, et al. A systematic review on endometriosis during pregnancy: diagnosis, misdiagnosis, complications and outcomes. *Hum Reprod Update* 2016, 22: 70–103.

32. Bulletti C, De Ziegler D, Polli V, Del Ferro E, Palini S, Flamigni C. Character- istics of uterine contractility during menses in women with mild to moderate endometriosis. *Fertil Steril* 2002, 77: 1156–61.

33. Kunz G, Beil D, Huppert P, Noe M, Kissler S, Leyendecker G. Adenomyosis in endometriosis–prevalence and impact on fertility. Evidence from magnetic resonance imaging. *Hum Reprod* 2005, 20: 2309–16.

34. Exacoustos C, Luciano D, Corbett B, De Felice G, Di Feliciantonio M, Luciano A, et al. The uterine junctional zone: a 3-dimensional ultrasound study of patients with endometriosis. *Am J Obstet Gynecol* 2013, 209: 248.e1–7.

35. Brosens JJ, Pijnenborg R, Brosens IA. The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *Am J Obstet Gynecol* 2002, 187: 1416–23.

36. Cha J, Sun X, Dey SK. Mechanisms of implantation: strategies for successful pregnancy. *Nat Med* 2012, 18: 1754–67.

37. Allerstorfer C, Oppelt P, Enzelsberger SH, Shamiyeh A, Schimetta W, Shebl OJ, et al. Delivery after operation for deeply infiltrating endometriosis. *Biomed Res Int* 2016, 2016: 8271452.

Chapter 7

Fertility, pregnancy and neonatal outcomes of patients with adenomyosis: a systematic review and meta-analysis

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Abstract

This study aimed to investigate the association of adenomyosis with fertility, pregnancy and neonatal outcomes. An electronic search was conducted using Medline, Pubmed and Cochrane databases up to April 2020. Seventeen observational studies were included. Adenomyosis was significantly associated with a lower clinical pregnancy (OR 0.69; 95% CI 0.51, 0.94) and higher miscarriage rate (OR 2.17; 95% CI 1.25, 3.79) after assisted reproductive technology (ART). The lower clinical pregnancy rate was more significant in the subgroup of patients with short downregulation protocols. After age-adjustment, similar associations were recorded. Adenomyosis was also significantly associated with an increased risk of preeclampsia, preterm delivery, Caesarean section, fetal malpresentation, small-for-gestational-age infancy and post-partum hemorrhage, which was confirmed after correction for age and mode of conception. In conclusion, adenomyosis is associated with negative effects on fertility after ART. The potentially protective role of the ultra-long downregulation protocols needs further evaluation in randomized controlled studies. Adenomyosis is also associated independently of the mode of conception with adverse pregnancy and neonatal outcomes. Proper counselling prior to ART and close monitoring of pregnancy in patients with adenomyosis should be recommended.

Introduction

Adenomyosis, characterized by the presence of endometrial epithelial and stromal cells within the myometrium, is an enigmatic gynecological disorder with an estimated prevalence of 20-35% [1, 2]. It is a heterogeneous disease, both in anatomical and clinical phenotype varying from normally sized to much enlarged uterus and from heavy dysmenorrhea and hypermenorrhea to no symptoms [3] while it is frequently coexistent with endometriosis [4].

Apart from the widely known implications of endometriosis on pain and quality of life, it has recently emerged that it may negatively influence pregnancy and neonatal outcomes [5-7]. The negative correlation remains even after previously surgically excised endometriosis [8].

Adenomyosis was also reported to be similarly associated with adverse fertility, pregnancy and neonatal outcomes in a recent systematic review [9]. However, this review did not include all eligible studies and no sensitivity analysis according to possible confounders such as the age, number of previous pregnancies, previous mode of delivery and co-existence of endometriosis was performed. Most importantly, pregnancy outcomes were not evaluated based on the method of conception, which represents another possible bias considering that ART is an independent risk factor for pregnancy complications [10] and that many pregnancies in patients with adenomyosis result only after ART. Finally, not only ART but also the stimulation protocol type may influence the outcomes [11].

Therefore, this study aimed to a) investigate the association of adenomyosis with fertility outcomes based on the stimulation protocol for the ART and adjusted to possible confounders, b) assess the association of adenomyosis with pregnancy and neonatal outcomes separately after natural and ART conception as well as adjusted to other possible confounders and c) determine if certain adenomyosis subtypes have a greater impact than others on the reproductive course.

Materials and Methods

We conducted a systematic search for all eligible studies in Medline, PubMed and Cochrane. Combinations of the terms “adenomyosis”, “pregnancy”, “fertility”, “neonatal outcomes” and “assisted reproductive technology” were used (((pregnancy) OR (fertility) OR (neonatal outcomes) OR (assisted reproductive technology)) AND (adenomyosis)). Studies published until 01.04.2020 in English language were included. Reference sections of all relevant studies, key journals and abstracts from the major annual meetings in the field were also searched. Inclusion criteria were: 1) controlled studies, where both cases (women with adenomyosis) and controls were assessed, 2) description of the method of the diagnosis of adenomyosis, 3) existence of data on fertility, pregnancy or neonatal outcomes. Data from sources other than original full publications (reviews, abstracts, oral presentations, national or local health statistics) were excluded. Two investigators (D-RK, KN) completed the main search and any discrepancy was solved by consultation of a third investigator, not involved in the initial procedure (MM).

Information from each study was extracted independently by two reviewers using a standardized data extraction form, which included general characteristics of the study (author, year of publication, country, design, number of patients and controls, method of assessment of adenomyosis diagnosis, matching factors, possible

confounders), clinical characteristics of the patients (age, mode of conception, co-existence of endometriosis) and requested outcomes. Disagreement was resolved by consensus. Where appropriate, the data set was completed through communication with the authors. Specifically, the authors of four studies were contacted [12-15] with one returning to us the requested raw data [12].

Included outcomes were: 1) fertility outcomes after ART (clinical pregnancy and live birth rate per cycle, miscarriage rate per pregnancy), 2) pregnancy/obstetrical outcomes (pre-eclampsia, pregnancy-induced hypertension, gestational diabetes, placenta praevia, placental abruption, small for gestational age infant (SGA; defined as birthweight <10th centile for gestational age), intrauterine growth retardation (IUGR), preterm and severe preterm delivery (gestational age <37 weeks and <32 weeks respectively), fetal malpresentation, Caesarean section delivery, operative vaginal delivery, intrauterine death, antepartum and postpartum hemorrhage (PPH), chorioamnionitis, uterine rupture, vaginal laceration, 3rd or 4th degree perineal or button hole tear), 3) neonatal outcomes (pHa, pHv, 5min Apgar score, admission to the neonatal unit for any reason (admission between birth and 28 days old), neonatal death (death between birth and 28 days old).

In order to assess the risk of bias, all studies were examined with the QUIPS tool [16]. The different types of bias, namely selection, performance, detection, attrition and reporting bias assessment was performed independently by two investigators (D-RK, KN). Any discrepancy was solved by consultation of an investigator, not involved in the initial procedure (MM).

Odds Ratio (OR) for dichotomous outcomes or mean differences (MD) for continuous outcomes and standard deviation (SD) were calculated for all studies included in the meta-analyses [17]. Heterogeneity among the outcomes of different studies was examined by the I2index [18], with I2 $\geq$ 50% indicating significant heterogeneity [19]. A random effects model and where applicable the inverse variance method was applied for every outcome [17]. Funnel plots, which graph OR on a log scale (effect) against standard error of log-OR (precision), were generated and visually inspected for asymmetry to determine if the included studies were non-representative of the body of possible studies on the subject (which could result from small study effect or other biases, such as publication and poor-quality bias). Statistical significance was set at a p-level of 0.05. Meta-analysis was conducted using Review Manager (RevMan) for Mac (version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). The report of the study was complemented in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) group standards for reporting meta-analysis of observational studies [20] and the protocol was submitted for registration in the PROSPERO international database for systematic reviews prior to data extraction but to date not yet registered (acknowledgement of receipt: 179366).

Results

The systematic research identified 561 articles. Seventeen observational studies met the inclusion criteria and were included in qualitative and quantitative analysis (Figure 1). Of these, four were prospective [21-24] and 13 retrospective studies [12-15, 25-33]. One prospective study was excluded since there was no precise definition of diagnosis of adenomyosis in MRI, although IVF outcome was categorized based on the uterine junctional zone thickness [34]. Adenomyosis was diagnosed by

transvaginal ultrasound (TVUS), magnetic resonance imaging (MRI) or both in 11 [15, 21, 22, 24-27, 29-32], one [23] and four studies [12-14, 28], respectively. In one study, information about the presence of adenomyosis was obtained from a self-reported health questionnaire [33]. In nine studies, the case group was matched with the control group for at least one confounder [12, 13, 15, 21, 23-25, 27, 30]. Statistical adjustments of certain outcomes of the study for possible confounders were performed in six studies [14, 23, 26, 29, 30, 33]. The study characteristics in detail are shown in Table 1. Risk of bias assessment for each study is presented in Table 2.

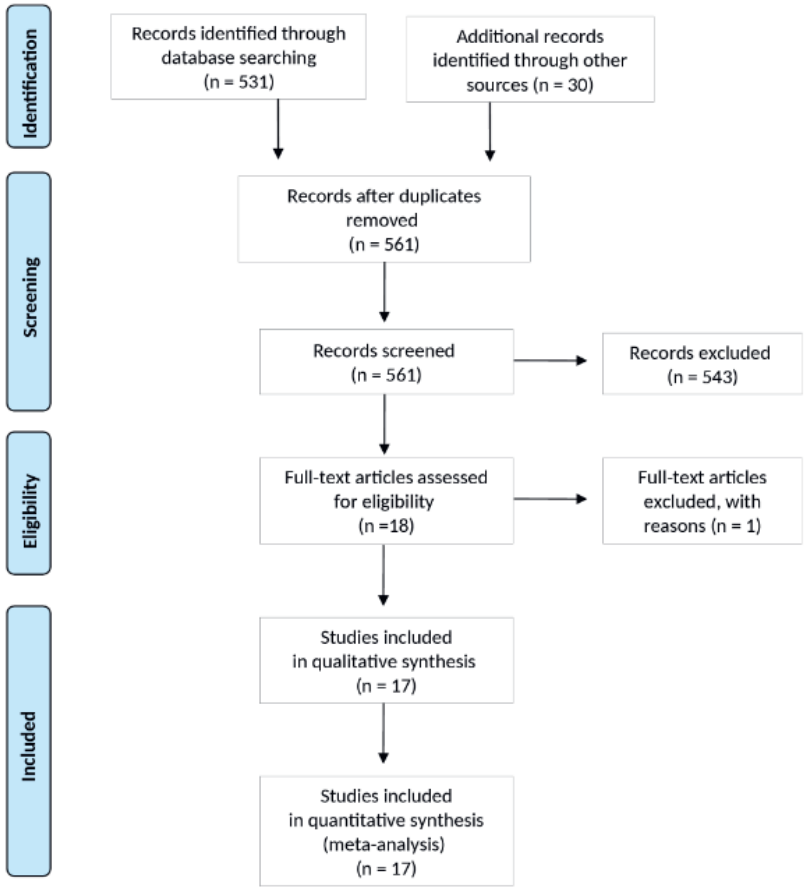


Figure 1. PRISMA 2009 Flow Diagram

Studies	Year	Country	Study design	Diagnosis Adenomyosis		adenomyosis			control				Potential confounders between case and control groups	conception	ART	
				diagnostical criteria	N	endometriosis (n)	characteristics		N	endometriosis (n)	characteristics	matching criteria			Protocol	No. of IVF cycles
Chiang et al.	1999	Taiwan	Single center prospective case-control study	TVUS	diffusely enlarged uterus without distinct uterine masses	19	NA	Infertility with enlarged uterus	144	NA	Infertile patients with normal uterus	age	No significant difference in age, no. of oocytes retrieved, no. of embryos transferred between groups	ART	short or long protocol (starting 10d before menstruation) with GnRHa	1
Juang et al.	2007	Taiwan	Single center retrospective case-control study	TVUS, MRI	MRI: i) a myometrial mass with indistinct margins of primarily low signal intensity with all sequences, ii) diffuse or focal widening (>0.5 cm) of the junctional zone on T2-weighted images, fast T2-weighted SE images, and con- trast material-enhanced T1-weighted images TVUS: i) thickening and asymmetry of the anterior and posterior myometrial walls, ii) increased echotexture of the myometrium,iii) heterogeneous, indistinctly marginated areas in the myometrium.	35	NA	Adenomyosis diagnosed on US (n=27), MRI (n=2) or both (n=6)	277	NA	Patients without adenomyosis during prenatal care	-	OR for preterm delivery adjusted for maternal age, parity, body mass index, smoking and status of previous preterm delivery. Not adjusted for mode of conception	ART, natural conception	n/a	n/a
Mijatovic et al.	2010	Netherlands	Single center retrospective cohort study	TVUS	At least 3 criteria: i) asymmetry of uterine walls without the presence of leiomyomas ii) myometrial areas of heterogenous echogenicity with poorly defined borders iii) minimal mass effect on the endometrium or the serosa which is relative to the size of the myometrial lesion iv) small myometrial cysts or hemorrhagic foci within the hetero-topic endometrial tissue v) echogenic nodules or linear striations radiating out from the endometrium into the myometrium vi) absence of circular vascularization (determined by colour Doppler) at the border of the lesion as usually observed with fibroids	20	20	Infertility in patients with adenomyosis and concomitant endometriosis; 18 patients with focal adenomyosis	54	54	Infertile patients with endometriosis without adenomyosis	endometriosis	No significant difference in rASRM stage; other possible confounders between groups not assessed, information not provided	ART	ultra-long protocol GnRHa (mean 5 months, range 3-26 months)	1
Costello et al.	2011	Australia	Single center retrospective cohort study	TVUS	Mandatory: i) subjective enlargement of the uterine corpus and ii) heterogeneity of myometrium/hypoechoic striations; Non-mandatory: i) asymmetrically thickened myometrium between anterior and posterior walls, (ii) myometrial cysts, (iii) poor definition of endometrial-myometrial junction.	37	5	Infertile patients with adenomyosis	164	16	Infertile patients without adenomyosis	--	No significant difference in year of treatment, time between TVS and IVF/ICSI treatment cycle, duration of infertility, body mass index, cause of infertility, gravidity, smoking, presence of endometriosis, IVF/ICSI treatment cycle number, presence of uterine fibroid(s). Patients with adenomyosis were older compared to patients without. OR for live birth rate and clinical pregnancy rate adjusted for age and IVF/ICSI.	ART	ultra-long downregulation protocol (oral contraceptive pill from day 5 of the previous menstrual cycle for 21 days; 15 days after starting the contraceptive start with GnRHa for at least 10 days)	1-3
Youm et al.	2011	Korea	Single center retrospective case-control study	TVUS	Not clearly defined: i) degree of uterine enlargement was classified according to myometrial thickness, ii) myometrial striations, heterogeneous myometrium, myometrial cysts, and poor definition of the endometrial–myometrial junction	81	NA	Infertile patients with adenomyosis	73	NA	Infertile patients without adenomyosis	maximum myometrial thickness	Due to study design unclear if difference in age, duration of infertility, no. of previous ART treatments, causes of infertility	ART	short GnRHa	1-2
Martinez Conejero et al.	2011	Spain	Single-center retrospective cohort study	TVUS, MRI	TVUS: hypoechogenic and heterogeneous areas with decreased echogenicity associated with elliptic intramyometrial lakes of more than 2 mm in diameter in a globular-appearing uterus MRI: areas of decreased signal intensity and a posterior junctional zone of more than 12 mm	152	23	Infertile patients with adenomyosis undergoing oocyte donation cycles	147	0	Infertile patients with normal ultrasonography, regular menses without endometriosis undergoing oocyte donation cycles	-	No significant difference in age, duration of infertility, indication of egg donation	ART	oocyte donation protocol with long GnRHa (leuprolide acetate in the secretory phase of the previous cycle)	1 - 3

Thalluri et al.	2012	Australia	Single-center retrospective cohort study	TVUS	i) enlarged uterus, ii) asymmetric thickening of the anterior or posterior myometrial wall, iii) heterogeneous poorly circumscribed areas within the myometrium, iv) anechoic myometrial blood filled lacunae or cysts of varying sizes, v) increased echo-texture of the endometrium and subendometrial linear striations	38	1	Infertile patients with adenomyosis	175	4	Infertile patients without adenomyosis	-	No significant difference in BMI, gravidy, parity, cause of infertility, endometriosis. Age and duration of infertility significantly higher in the case group. OR for live pregnancy rate adjusted for age.	ART	GnRH antagonist protocol	1
Salim et al.	2012	United Kindom	Single-center prospective cohort study	TVUS	All of the following criteria: i) asymmetrical thickening of the myometrium and ii) irregular cystic areas within the myometrium and iii) linear striations radiating out from the myometrium	19	1	Infertile patients with adenomyosis	256	21	Infertile patients without adenomyosis	-	No significant difference in the age of women, FSH, cause and duration of infertility, body mass index, total dose of gonadotrophin used and number of oocytes collected. Women with adenomyosis had a significantly higher mean antral follicle count compared with women with a normal uterus	ART	long protocol GnRHa (starting in the previous mid-luteal phase)	1
Ballester et al.	2012	France	Multicenter prospective study	MRI	(i) maximal junctional zone thickness (JZmax) of at least 12 mm, (ii) ratiomax (JZmax/myometrial thickness) >40% and (iii) punctate high-density myometrial foci	21	21	Infertile patients with colorectal endometriosis and adenomyosis	54	54	Infertile patients with colorectal endometriosis but without adenomyosis	colorectal endometriosis	Age, BMI and parity unmatched; unlear if different between groups; multivariate analysis for clinical pregnancy rate per patient with adjustment for age and AMH. Clinical pregnancy rate per cycle cannot be extracted.	ART	long (starting in the previous mid-luteal phase) and short protocol GnRHa, antagonist protocol	1 - 3
Yan et al.	2014	China	Single-center retrospective cohort study	TVUS	Mandatory: i) heterogeneous areas in the myometrium with poorly defined borders; enhanced by: ii) presence of clinical symptoms such as dysmenorrhea and irregular uterine bleeding or surgical pathology reports	77	21	Infertile patients with adenomyosis	77	11	Infertile patients without adenomyosis	age, BMI, gravidy, cause of infertility, type of stimulation protocol	Case group with 27.3% concomitant endometriosis vs 14.3% in the control group. OR for all outcomes adjusted for mean day 3 estradiol, total dosage of gonadotropin per cycle, duration of gonadotropin stimulation, endometriosis.	ART	short, long and ultralong protocol with GnRHa	1
Benaglia et al.	2014	Italy	Single-center prospective case-control study	TVUS	i) asymmetrical thickening of the anterior and posterior walls of myometrium or ii) irregular cystic areas or iii) linear striations radiating out from the myometrium or iv) irregular endometrial-myometrial junction	49	21	24 infertile patients with focal adenomyosis, 25 with diffuse adenomyosis (long GnRHa: 53%)	49	13	Infertile patients without adenomyosis	age, study period, day of embryo transfer and number of transferred embryos	No significant differrence in type of stimulation protocol. Patients with endometriosis 43% in the case vs 27% in the control group	ART	long and short protocol with GnRHa, antagonist protocol	1
Mochimaru et al.	2015	Japan	Single-center retrospective case-control study	TVUS, MRI	MRI: i) a myometrial mass with indistinct margins of primarily low intensity, ii) diffuse or local widening of junctional zones on T2-weighted images. TVUS: i) myometrial anterior posterior asymmetry, ii) thickening of the anterior and posterior myometrial walls, with either increased or decreased echogenicity	36	NA	Pregnancies of women with adenomyosis	144	NA	pregnancies of women without uterine anomalies in ultrasonography in the first trimester	age	No significant difference in BMI, history of miscarriage. Significant differences in nulliparity, need for ART.	ART, natural conception	n/a	n/a
Hashimoto et al.	2018	Japan	Multicenter retrospective case-control study	TVUS, MRI	MRI: i) a myometrial mass with indistinct margins of primarily low signal intensity or ii) diffuse or focal thickening of the junctional zone forming an ill-defined area of low signal intensity on T2-weighted images. TVUS: i) thickening or asymmetry of the anterior and posterior myometrial walls, with either increased or decreased echogenicity or ii) increased echotexture of the myometrium or iii) heterogeneous, indistinctly marginated areas in the myometrium	49	NA	Pregnancies of women with adenomyosis (46.9% ART)	245	NA	pregnancies of women without adenomyosis in the first trimester (46.9% ART)	age, parity, need for ART	No data about endometriosis.	ART, natural conception	n/a	n/a

Shin et al.	2018	Korea	Multicenter retrospective cohort study	TVUS	Two or more of the following: i) a globular or asymmetric uterus, ii) a poorly defined heterogeneous myometrium, iii) a distorted and heterogeneous myometrial echo texture, iv) irregular myometrial cystic lesions, v) hypoechoic linear striations, vi) an irregular endometrial-myometrial junction	25	NA	Pregnancies of women with adenomyosis (34.7% ART)	187	NA	pregnancies of women without US findings of adenomyosis in early pregnacy (2.3% ART)	-	No significant difference in age, nulliparity, previous miscarriage. Higher Nr of pregnacies in case group after ART aber data can be extracted separately. No data about endometriosis.	ART, natural conception	n/a	n/a
Sharma et al.	2018	India	Single-center retrospective cohort study	TVUS	At least 3 of the following: i) increased myometrial thickness, ii) symmetrically thickened anterior or posterior myometrial wall, iii) poorly defined endo-myometrial interface, iv) presence of heterogeneous myometrial area, v) myometrial cysts	152	88	Infertile patients with adenomyosis	821	355	Infertile patients with tubal factor or endometriosis	-	No signiicant difference in age, BMI	ART	ultra-long protocol GnRHa (leuprolide acetate 3.75 mg in three doses every 28d)	1
Yamaguchi et al.	2018	Japan	Retrospective nationwide cohort study	self-reported questionnaire	yes/no	311	128	Pregnancies of women with self-reported adenomyosis (19.6% ART)	93210	3412	pregnancies of women without adenomyosis (3% ART)	-	Age, rate of primiparity, rate of ART significantly higher in case group. Risk of preterm birth, low birthweight and SGA adjusted for maternal age, smoking status, method of conception, primiparity, coexistence of fibroids or endometriosis, BMI	ART, natural conception	n/a	n/a
Scala et al.	2018	Italy	Retrospective cohort study	TVUS	At least two criteria: i) asymmetrical myometrial thickening, ii)myometrial cysts, iii) linear striations, iv) hyperechoic islands or v)an irregular and thickened endometrial–myometrial junctional zone on either two- or three-dimensional imaging	58	58	Women with Adenomyosis; 20 diffuse and 38 focal adenomyosis with 20% and 15.8% ART, respectively	148	148	Pregnancies of women with histolologic al or US diagnosis of endometriosis with 12.8% ART	endometriosis	Patients with diffuse adenomyosis had significantly lower BMI. No significant difference in age, rate of ART, previous miscarriage, smoking status, type of endometriosis	ART, natural conception	n/a	n/a

Table 1. Study characteristics

Paper / Tool sectors	Study Participation	Study Attrition	Prognostic Factor Measurement	Outcome Measurement	Study Confounding	Statistical Analysis and Reporting	Overall assessment
Chiang et al, 1999	M	L	M	L	M	L	**M**
Juang et al., 2006	M	L	M	L	L	L	**M**
Mijatovic et al., 2010	M	L	L	L	H	L	**M**
Costello et al., 2011	L	L	L	L	L	L	**L**
Youm et al., 2011	M	L	M	L	M	L	**M**
Martinez Conejero et al., 2011	M	L	M	L	L	L	**M**
Thalluri et al., 2012	M	L	M	L	L	L	**M**
Salim et al., 2012	M	L	L	L	L	L	**L**
Ballester et al., 2012	H	L	L	L	L	L	**M**
Yan et al., 2014	L	L	L	L	L	L	**L**
Benaglia et al., 2014	L	L	L	L	L	L	**L**

Mochimaru et al., 2015	M	L	M	L	M	L	**M**
Hashimoto et al., 2017	M	L	M	L	L	L	**M**
Shin et al., 2018	M	L	M	L	L	L	**M**
Sharma et al., 2018	L	L	L	L	L	L	**L**
Yamaguchi et al., 2018	H	L	H	L	L	L	**H**
Scala et al., 2018	L	L	L	L	M	L	**L**

L: Low risk; M: Moderate risk; H: High risk

Table 2. Risk of bias

Fertility outcomes after ART

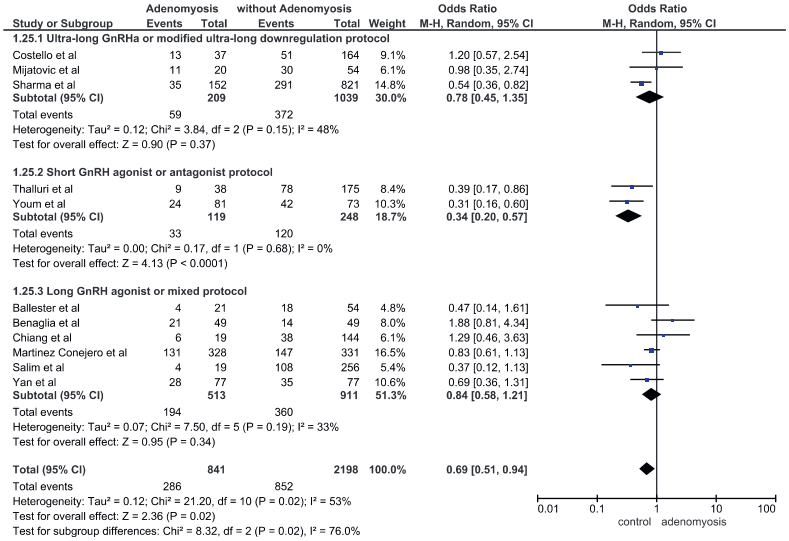
We separated eligible studies in subgroups according to the ART protocol. The first subgroup included only the studies with an ultra-long [25, 32] or modified ultra-long [26] pituitary downregulation protocol. The second subgroup included only the studies with a short downregulation protocol. Specifically, a GnRH antagonist and a GnRHa short protocol was applied in all patients in the [29] and [27] study, respectively. The third group was a mixed group including studies with long GnRHa protocol (starting in the previous mid-luteal phase) and studies with more than one protocol [21-24, 28, 30]. The meta-analysis of the first subgroup showed no significant difference in clinical pregnancy rate (3 studies, 209 vs. 1039 women, OR 0.78; 95% CI 0.45, 1.35), live birth rate (2 studies, 189 vs. 985 women, OR 0.64; 95% CI 0.19, 2.14) and miscarriage rate (3 studies, 68 vs. 404 pregnancies, OR 1.23; 95% CI 0.31, 4.91) (Figure 2). The meta-analysis of the second subgroup showed a significantly lower clinical pregnancy rate (2 studies, 119 vs. 248 women, OR 0.34; 95% CI 0.20, 0.57) and higher miscarriage rate in the adenomyosis group (2 studies, 36 vs. 129 pregnancies, OR 4.32; 95% 1.77, 10.55), while only one study provided data for the live birth rate showing a significantly lower value in the adenomyosis group (1 study, 81 vs. 73 women, OR 0.19 95% CI 0.09, 0.42) (Figure 2). The third subgroup (long GnRH agonist or mixed protocol) showed no difference in clinical pregnancy rate (6 studies, 513 vs. 911 women, OR 0.84; 95% CI 0.58, 1.21) and live birth rate (3 studies, 145 vs. 270 women, OR 0.82; 95% CI 0.27, 2.49) between the two groups but close to significant higher miscarriage rate in the adenomyosis group (5 studies, 410 vs. 643 pregnancies, OR 2.30; 95% 0.98, 5.39) (Figure 2).

When all studies were combined a significantly lower clinical pregnancy rate (11 studies, 841 vs. 2198 women, OR 0.69; 95% CI 0.51, 0.94) and significantly higher miscarriage rate (10 studies, 514 vs. 1176 women, OR 2.17; 95% 1.24, 3.80) was shown in the adenomyosis group. No significant difference in live birth rate (6 studies, 415 vs. 1328 women, OR 0.58; 95% CI 0.29, 1.17) was found (Figure 2).
Sensitivity analysis. Since age is a major confounder for the fertility outcome, studies in which age was significantly different or of unknown difference between the case and control group were excluded unless the fertility outcome was reported age-adjusted. In the latter case, the adjusted ORs were used for the meta-analysis via inverse variance method. The same trends as in the crude analysis were observed. The result for clinical pregnancy rate was marginally non-significant (8 studies, OR 0.78; 95% CI 0.58, 1.05) (Figure 2B). Miscarriage rate was found significantly higher in the adenomyosis group (6 studies, OR 2.50; 95% CI 1.26, 4.95).

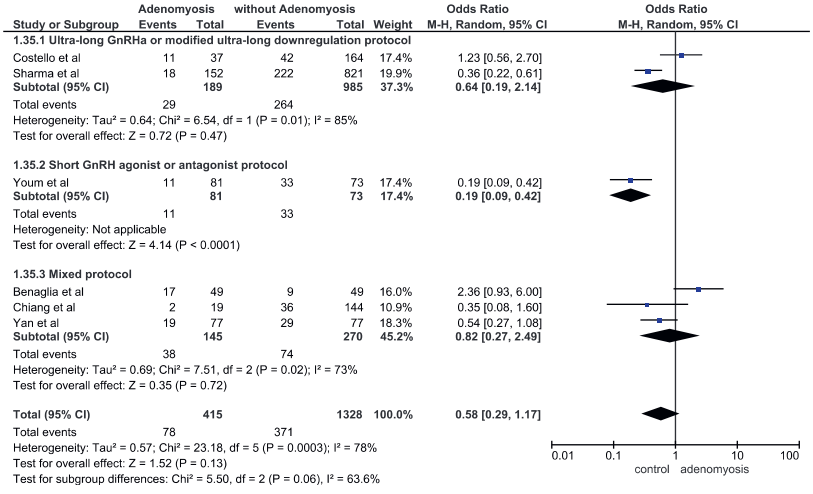
In a second sensitivity analysis, only studies with matched groups for endometriosis were included in order to evaluate the association of adenomyosis with fertility outcomes independently of endometriosis. Two studies could be included with the first excluding endometriosis from all patients [32] and the other having endometriosis in all patients [25]. In both studies, an ultra-long downregulation protocol for ART was applied. The results were non-significant for both clinical pregnancy and miscarriage rates (Figure 2C).

A

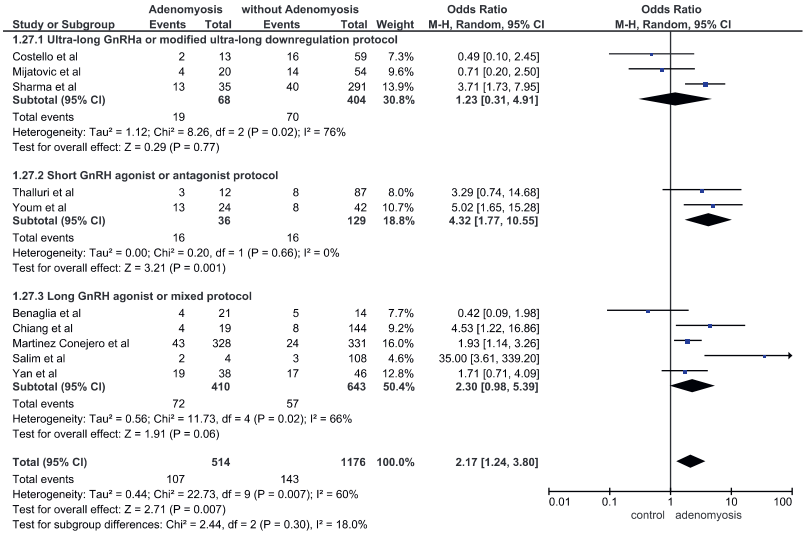
Clinical pregnancy rate



Live birth rate

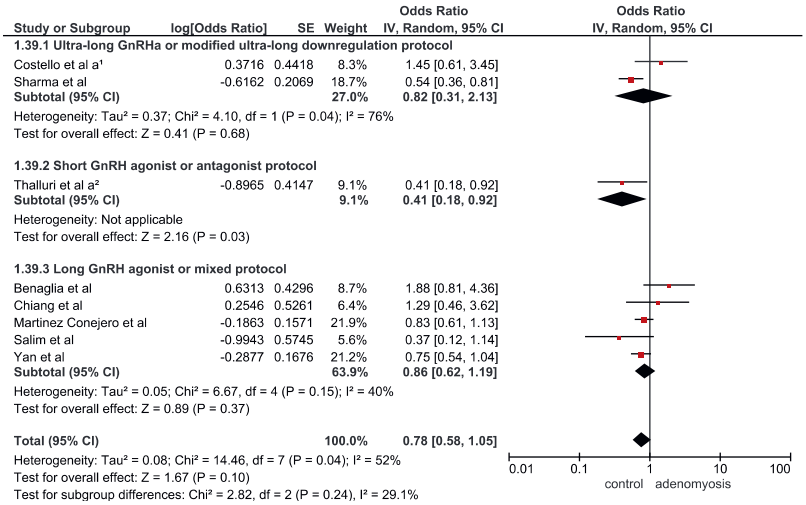


Miscarriage rate

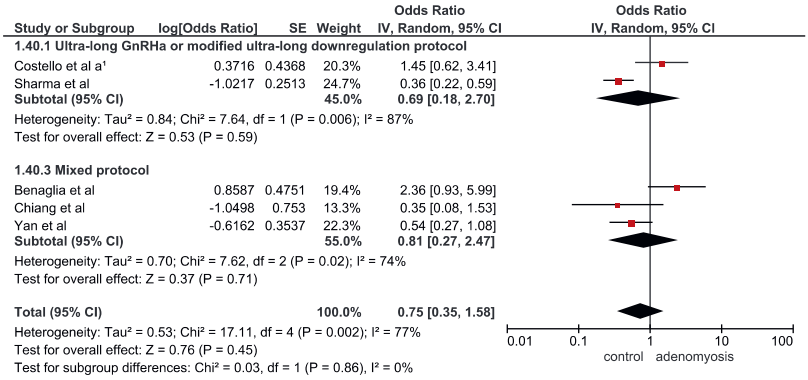


B

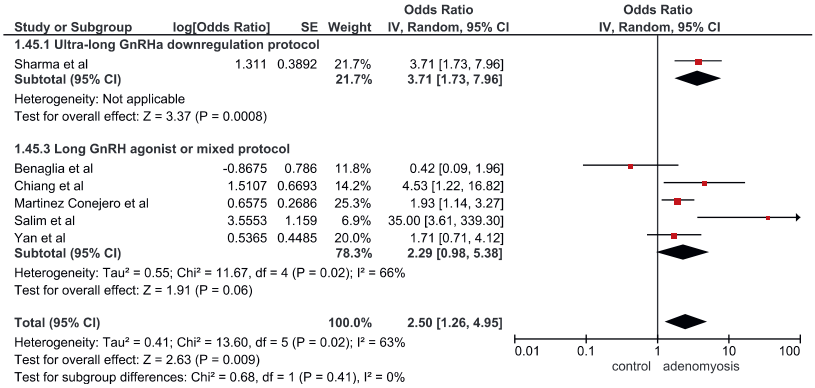
Clinical pregnancy rate



Live birth rate

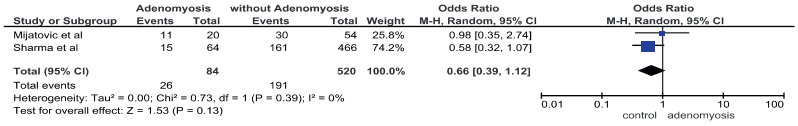


Miscarriage rate



C

Clinical pregnancy rate



Miscarriage rate

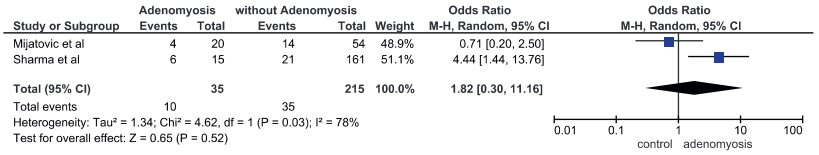


Figure 2. Fertility outcomes
A. Crude rates
B. Sensitivity analysis. Age-adjusted. Studies with unknown or significant age difference between groups were excluded. Note: a1 and a2 adjusted for maternal age
C. Sensitivity analysis. Studies with matched groups for endometriosis.

Pregnancy outcomes

Preterm delivery (< 37 weeks)

Six studies provided data on this outcome. Two of them presented significant age differences between groups but the outcome could be age-adjusted [14,33]. Another two were primarily matched for age [12,13] and one presented no significant age differences between groups [31]. The meta-analysis showed a statistically significant higher risk of preterm delivery in the adenomyosis group (6 studies, OR 2.65; 95% CI 2.07, 3.39), (Figure 3A).

Sensitivity analysis. This was performed only in studies which were matched or adjusted for the mode of conception (natural or ART). Since the rate of preterm delivery could not be adjusted in the study by Juang et al, it was excluded [14]. The meta-analysis of all studies showed a significantly higher risk of preterm birth in the adenomyosis group (5 studies, OR 2.83: 95% CI 2.18, 3.69). In Figure 3B, the results are presented separately according to the mode of conception. Briefly, the difference remains statistically significant both for ART and natural pregnancies.
Only one study [33] provided the risk of preterm delivery adjusted for endometriosis. It showed that the increased risk of preterm delivery remained in the adenomyosis group (OR 2.49; 95% CI 1.81, 3.43).

Severe preterm delivery (< 32 weeks)

The meta-analysis of two studies [12, 32] showed no significant difference (2 studies, 58 vs. 395 women; OR 2.20; 95% 0.82, 5.89), (Figure 3C).

Preeclampsia

Four studies provided data for this outcome. Two were primarily matched for age[12,13] and two presented no significant age differences between groups [15,32]. The meta-analysis showed a significantly higher risk for women with adenomyosis (4 studies, 159 vs. 785 women, OR 4.32; 95% CI 1.68, 11.09). In Figure 3D, the results are presented separately according to the mode of conception.

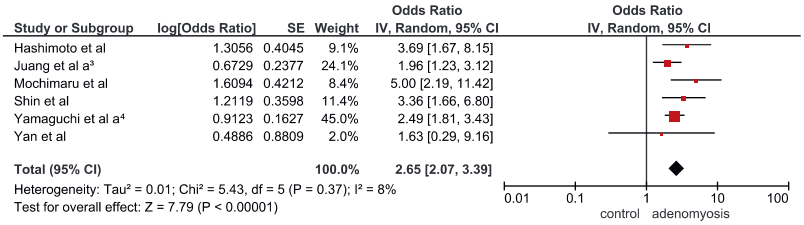
Pregnancy-induced hypertension

The only study with this available outcome [13] showed a higher risk in the adenomyosis group (43 vs. 242 women, OR 3.11; 95% CI 1.10, 8.79).

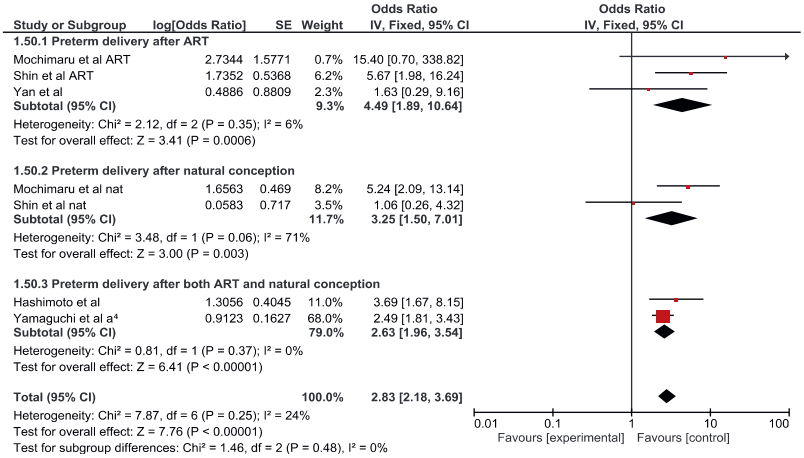
Gestational diabetes

The only study with this available outcome [13] showed a higher risk in the control group (43 vs 242 women, OR 0.11; 95% CI 0.02, 0.85).

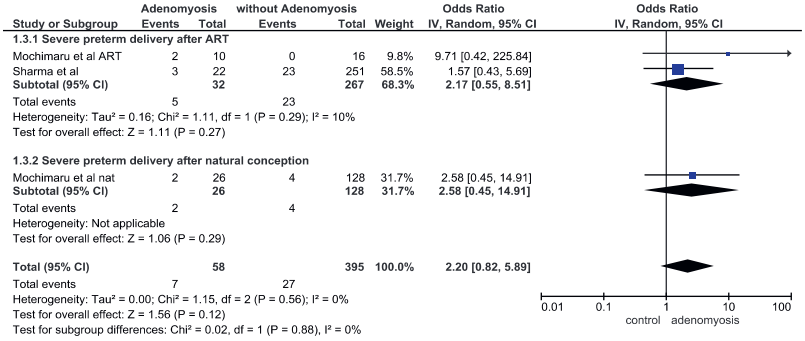
A. Preterm delivery



B. Preterm delivery. Sensitivity analysis



C. Severe preterm delivery



D. Preeclampsia

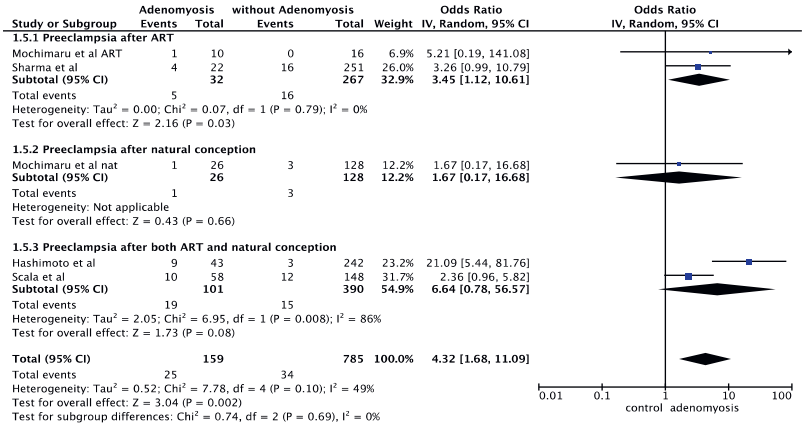


Figure 3. Pregnancy outcomes
A. Preterm delivery (age adjusted)
Note: No significant difference in age in the study by Shin et al. (no adjustment performed). In all other studies age matched between groups.
a[3]:adjusted for maternal age, parity, body mass index, smoking habit, and status of previous preterm delivery
a[4]:adjusted for maternal age, smoking status, method of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy
B. Preterm delivery. Sensitivity analysis (age- and mode of conception-adjusted). Studies with unknown or significant difference in age or mode of conception between groups were excluded
Note: No significant difference in age in the study by Shin et al. (no adjustment performed). In all other studies age matched between groups.
a4: adjusted for maternal age, smoking status, method of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy
C. Severe preterm delivery
Note: No significant difference in age in the study by Sharma et al. (no adjustment performed). In the study by Machimaru et al. age matched between groups.
D. Preeclampsia (age- and method of conception- adjusted)
Note: No significant difference in age in the study by Sharma et al. No significant difference in age and mode of conception in the study by Scala et al. However, for both studies no adjustment performed.

Obstetrical outcomes

Caesarean section, fetal malpresentation

Meta-analysis of all available studies showed a significantly higher risk of Caesarean section in the adenomyosis group (4 studies, 462 vs. 101840 women; OR 2.48; 95% CI 1.44, 4.26) (Figure 4A). In a sensitivity analysis, one study [33] was excluded due to significantly higher age and rate of ART in the case group, which could not be adjusted. Shin et al. [31] was also excluded due to significantly higher rate of ART in the case group. The meta-analysis of the remained two studies (12, 13), uninfluenced from the age and mode of delivery, showed a higher risk of

Caesarean section in the adenomyosis group (2 studies, 79 vs. 386 women; OR 4.44; CI 2.64, 7.47), (Figure 4B). The same two studies showed that women with adenomyosis have a higher risk of fetal malpresentation (2 studies, 79 vs. 386 women, OR 3.05; 95% CI 1.60, 5.81) (Figure 4C).

Operative vaginal delivery

Mochimaru et al. [12] was the only study providing data on this outcome. No statistically significant difference between the two groups was shown (10 vs. 16 women after ART pregnancy; OR 0.49; 95% CI 0.02, 13.28), (26 vs. 128 women after natural conception; OR 1.24; 95% CI 0.13, 11.57).

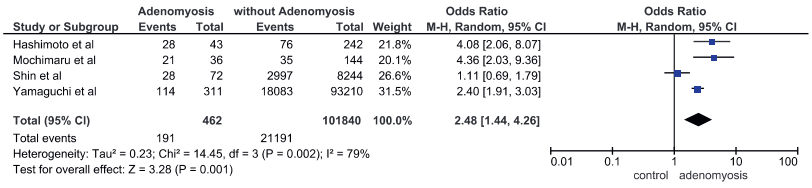
Postpartum, antepartum hemorrhage

Concerning PPH, the meta-analysis showed a significantly higher risk in the adenomyosis group (3 studies, 101 vs. 637 women; OR 2.90; 95% CI 1.39, 6.05) (Figure 4D). A single study [32] reported no significant difference in antepartum hemorrhage between the two groups (22 vs. 251 women; OR 0.66; 95% CI 0.08, 5.17).

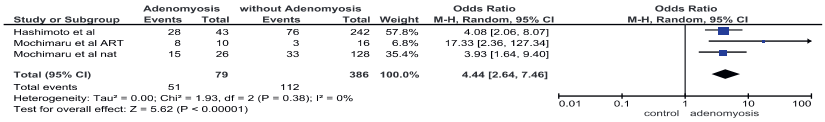
Placental malposition

Only one study [13] presented data on placental malposition showing a significantly higher risk in the adenomyosis group (49 vs 245 women, OR 4.94; 95% 1.70, 14.34).

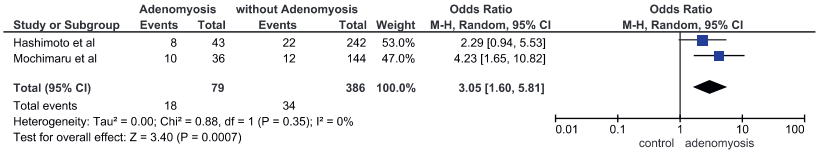
A) C-section



B) C-section. Sensitivity analysis



C) Fetal malpresentation



D) Postpartum Hemorrhage

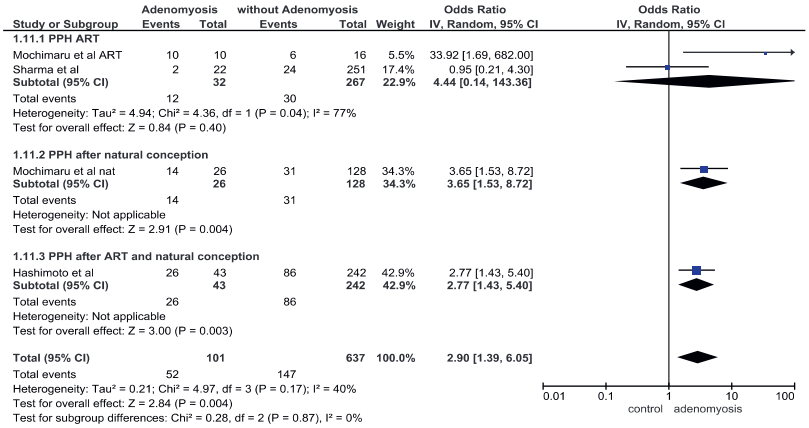


Figure 4. Obstetrical outcomes
A. C-section
Note: In the study by Yamaguchi et al. significantly higher age and rate of ART in the adenomyosis compared to control group (not adjustable). In the study by Shin et al. significantly higher rate of ART in the adenomyosis compared to control group (not adjustable). In the study by Hashimoto groups matched for age and rate of ART.
B. C-section. Sensitivity analysis (Only studies matched for age and rate of ART)
C. Fetal malpresentation
Note: In both included studies groups matched for age.
D. Postpartum Hemorrhage
Note: In all studies groups matched for age or not significantly different. In the study by Hashimoto et al. groups matched for age and rate of ART. Mochimaru et al. data was provided by the author (not published).

Neonatal outcomes

Small for gestational age (SGA)

Four studies provided data on this outcome. The groups in the study by Hashimoto [13] were primarily matched for age and rate of ART. Similarly, the study by Mochimaru [12] was primarily matched for age while, after provision of their raw data, the outcome could be extracted separately according to the mode of conception. Yamaguchi et al [33] provided the outcome adjusted for age and mode of conception and finally the groups in the study by Scala et al [15] were not significantly different for age and mode of conception. A meta-analysis of these four studies showed a significantly higher risk in the adenomyosis group (4 studies, OR 2.86, 95% CI 1.68, 4.88), (Figure 5A). In sensitivity analysis, only studies matched for endometriosis were included. Two studies showed that the risk of SGA in patients with endometriosis remained significantly higher (2 studies, OR 2.10; 95% CI 1.17, 3.77), (Figure 5B).

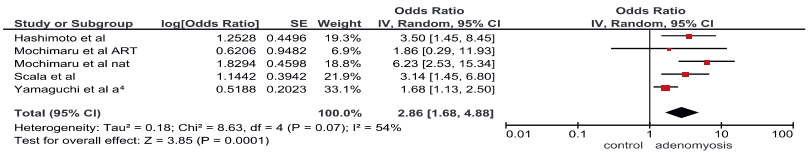
Low birth weight (LBW)
A meta-analysis of two studies (31, 33) including both natural and ART pregnancies showed a higher risk of birth weight < 2500g (2 studies, OR 2.82, 95% CI 1.20, 6.62) and <1500g (2 studies; OR 5.67; 95% CI 0.91, 35.34) in the adenomyosis group. Shin et al. examined the risk of low birth weight (<2500g) separately in pregnancies after ART and natural conception. In ART pregnancies a significantly higher risk in the adenomyosis group was shown (25 vs. 187 women; OR 7.69; 95% CI 2.56, 23.10). However, in natural conception pregnancies no significant difference between groups was found (47 vs. 8057 women; OR 2.16; 95% CI 0.67, 6.96), (Figure 5C and D).

Intrauterine growth restriction (IUGR)
Only one study [32] presented data on this outcome showing a significantly higher risk in the adenomyosis group (22 vs. 251 women; OR 3.40; 95% CI 1.13, 10.17).

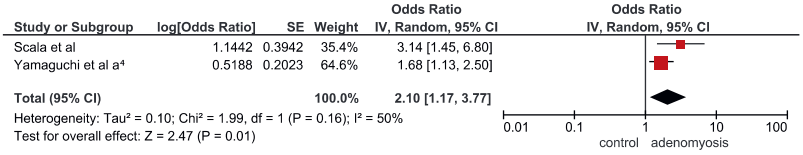
Intrauterine fetal death
The meta-analysis of two studies [12,32] showed no difference between the two groups (2 studies, 58 vs. 395 women; OR 1.43; 0.34, 6.04), (Figure 5E).

APGAR 5 min < 7, pHa, NICU admission
The meta-analysis of three studies [12,13,15] showed no significant difference in the rate of APGAR 5 min < 7 between groups (3 studies, 136 vs 534 women; OR 1.63; 0.56, 4.70), (Figure 5F). Mochimaru et al. [12] showed no difference in the rate of pHa< 7.1 between the two groups (36 vs 144 women, OR 4.4; 95% CI 0.5, 32.5). NICU admission rate according to the same study was higher in infants of women with adenomyosis compared to the control group (36 vs 144 women, OR 2.4; 95% CI 0.9, 6.3).

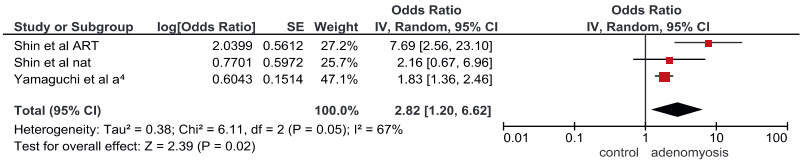
A. Small for gestational age (SGA)



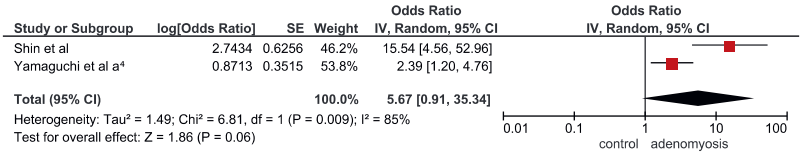
B. Small for gestational age. Sensitivity analysis



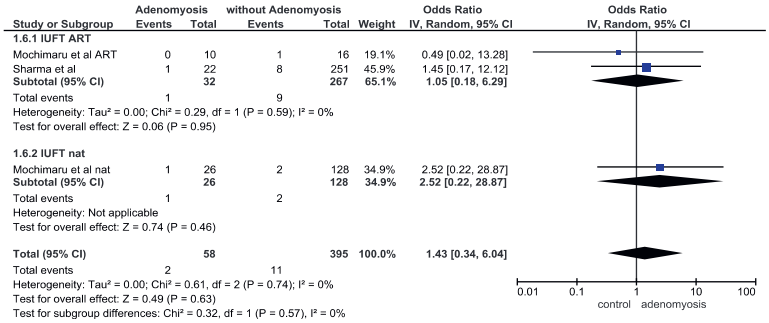
C. Birth weight < 2500g



D. Birth weight < 1500g



E. Intrauterine fetal death



F. APGAR 5 min < 7

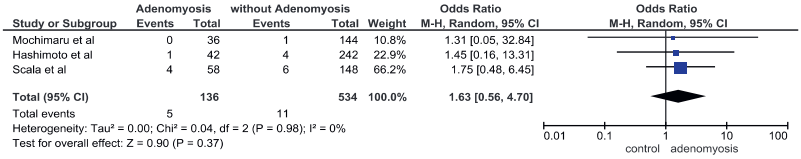


Figure 5. Neonatal outcomes
A. Small for gestational age (SGA) (adjusted for age and mode of conception)
Note: In the study by Hashimoto groups matched for age and mode of conception. In the study by Mochimaru groups matched for age and data presented separately according to the mode of conception. No significant difference in age and mode of conception in the study by Scala et al.
a[4]: Adjusted for maternal age, smoking status, mode of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy
B. Small for gestational age. Sensitivity analysis (Included studies matched for endometriosis)
Note: a[4]: Adjusted for maternal age, smoking status, mode of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy
C. Birth weight < 2500g
Note: a[4]: Adjusted for maternal age, smoking status, method of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy. Data in the study by Shin et. al presented separately according to the mode of conception
D. Birth weight < 1500g
Note: a[4]: Adjusted for maternal age, smoking status, method of conception, primiparity, fibroids, endometriosis, and body mass index before pregnancy. Data in the study by Shin et. al could not be extracted according to the mode of conception
E. Intrauterine fetal death
Note: In the study by Mochimaru groups matched for age and data presented separately according to the mode of conception. In the study by Sharma groups not significantly different for age.
F. APGAR 5 min < 7
Note: In the study by Hashimoto groups matched for age and rate of ART. In the study by Mochimaru groups matched for age. In the study by Scala groups not significantly different for age.

Effect of certain adenomyosis subtypes on the reproductive course

Two studies examined the effect of focal and diffuse adenomyosis separately, however on different outcomes. The type of adenomyosis did not markedly affect the fertility outcome in the study by Benaglia et al [24]. Specifically, the clinical pregnancy rate in women with focal (n = 24) and diffuse (n = 25) adenomyosis was 46% and 40%, respectively. The live birth rate was 33% and 36%, respectively. The implantation rate was 32% and 32%, respectively. In the study by Scala et al. 38 patients had focal and 20 diffuse adenomyosis[15]. Patients with diffuse adenomyosis had a higher rate of SGA births compared to the control group. This was not confirmed in patients with focal adenomyosis.

The funnel plots showed no indication of asymmetry in any of the analyses (Supplementary Figure 1).

Discussion

Principal findings

With respect to fertility outcomes, this systematic meta-analysis revealed that adenomyosis is associated with a significantly lower clinical pregnancy rate and higher miscarriage rate after ART, especially when a short GnRH agonist or antagonist protocol is administered for ovarian stimulation. Moreover, adenomyosis is associated with a higher risk of preterm delivery, preeclampsia, caesarean section, fetal malpresentation, SGA, low birth weight, and PPH. The association could be confirmed after adjustment of these outcomes for age and mode of conception.

Results in the context of what is known

A recent systematic meta-analysis evaluated various fertility outcomes in patients with adenomyosis concluding that the clinically pregnancy rate was reduced [9]. However, several studies were not included and no subgroup analysis for potential

confounders was performed, despite significant study heterogeneity. Previous studies suggested that the protocol for ovarian stimulation may be crucial for the fertility outcome in patients with adenomyosis [29]. Ultra-long GnRHa protocols produce a period of estrogen deficiency that may temporarily inactivate adenomyosis, reduce the uterine volume and normalize some of the distorted endometrial functions [29, 35]. This period of potentially therapeutic estrogen deficiency does not occur in GnRH antagonist or short GnRHa cycles. By separately meta-analyzing the ART studies based on the stimulation protocol we show that adenomyosis has a higher negative impact on the pregnancy rate when short downregulation protocols are applied. The same results were found after a sensitivity analysis, in which the fertility outcomes were adjusted for age, an otherwise major confounder for fertility outcomes.

One study [26] used a combined oral contraceptive (COC) pill for 21 days prior to a start with GnRHa for at least 10 days. Although this may not be regarded as an ultra-long ovarian downregulation, a COC-mediated effect on the endometrium occurs, which may be important in patients with adenomyosis. Therefore, we regarded this as a modified ultra-long downregulation and meta-analyzed it together with the other two studies which have applied a classical ultra-long GnRHa pretreatment [25, 32]. The results of the two studies with the classical ultra-long GnRHa pretreatment were discordant concerning its protective role in adenomyosis.

Moreover, the results have to be interpreted with caution since the control groups with ultra-long downregulation protocols show a surprisingly lower clinical pregnancy rate (3 studies, 372/1039, 35.8%) than the control groups with short downregulation protocols (2 studies, 120/248, 48.4%). The difference in the control groups may imply different populations or even a negative impact of the ultra-long GnRHa treatment on the pregnancy rate per cycle in control patients. Nevertheless, the positive effect of the ultra-long GnRHa pretreatment before ART in patients with adenomyosis is further supported by two retrospective controlled studies comparing GnRHa pretreatment versus no treatment before fresh–embryo [36] and frozen-embryo transfer [37]. Currently two RCT protocols are registered in the U.S. National Library of Medicine aiming at conclusively elucidating this issue (University College London, 2019; University Hospital Toulouse, 2019).

If the positive effect of prolonged GnRHa prior to ART on fertility outcome is confirmed, concerns about the excessive ovarian suppression, especially in women with reduced ovarian reserve, still need to be addressed. Given recent advances in vitrification technology resulting in enhanced embryo survival and pregnancy rates [38], it could be plausible to firstly vitrify embryos at the blastocyst stage to confirm adequate development and then administer prolonged GnRHa before endometrial preparation with the aim to inactivate adenomyosis, reduce the uterine volume and maybe normalize some of the distorted endometrial functions. This would eliminate concerns regarding excessive ovarian suppression without affecting any possible beneficial impact on fertility outcomes provided by GnRHa. However, one has to keep in mind that frozen blastocyst transfers are associated with a higher risk of preeclampsia (RR 3.13, 95% CI 1.06–9.30, p=0.029) [39]. This risk has to be weighed against the potential benefits of the above approach. Finally, it is currently unknown if alternative treatments with less side effects such as progestins could also be beneficial prior to ART.

Of particular interest is the study by Martínez et al [28], which included only patients with adenomyosis receiving donated oocytes. Interestingly, despite no difference in the pregnancy rate a higher risk of miscarriage was reported indicating a detrimental effect of adenomyosis on the reproductive outcome irrespective of the embryo quality.

A previous meta-analysis by Younes et al concluded that adenomyosis is not only correlated to reduced pregnancy rate but also reduced live birth rate[40]. Our results do not confirm the statistical significance of the latter although one additional study supportive for that was included [32]. The reason is that Younes at al. used a fixed effect model rather than random effects model for the meta-analysis, which consequently decreases the width of the confidence intervals. We chose to use a random effects model since we assumed a high study heterogeneity suggesting that the true effect size is not the same in all studies [41]. However, since adenomyosis is statistically significantly associated with both lower pregnancy and higher miscarriage rate it is plausible that it should also be associated with a statistically significantly lower live birth rate. We assume that the lack of statistical significance can be attributed to the lower statistical power resulting from the lower number of studies evaluating this outcome.

Horton et al performed the only meta-analysis to date which evaluated pregnancy outcomes in patients with adenomyosis [9]. However, in many studies the case compared to the control groups included a much higher number of ART pregnancies. Specifically, in the study by Shin et al 34% vs 2.3% of the pregnancies resulted after ART in the case and in the control group, respectively [31]. Similarly, Mochimaru et al included 33% vs 13% ART pregnancies in the case and in the control group, respectively [12]. Other included studies did not provide information on the mode of conception but the example of the previous studies and the knowledge that adenomyosis is correlated with subfertility [42, 43] suggest that the case groups may similarly include significantly more ART pregnancies and thus being unbalanced to the control groups. Since ART pregnancies are anyway at a higher risk of adverse pregnancy outcomes such as preeclampsia, preterm birth and low birth weight [10, 44], it is uncertain if the observed adverse outcomes should be attributed to adenomyosis or are just the effect of the ART conception. All outcomes of the current study were therefore analyzed according to the mode of conception and adjusted to age when possible. Interestingly, the increased risk of preterm delivery, preeclampsia, SGA, low birth weight and PPH in patients with adenomyosis persisted. We postulate, therefore, that at least for these pregnancy complications adenomyosis represents a significant risk factor independently of the mode of conception.

The increased risk of Caesarean section in patients with adenomyosis should be interpreted with caution due to the following reasons. The biggest study was based on a patient-reported questionnaire collected during the pregnancy for the diagnosis of adenomyosis while a significant difference in age, primiparity and sterility treatment between groups was reported [33]. The lack of matching for possible confounders applies partially to the other studies as well. This raises doubts about the independent association between adenomyosis and the risk of Caesarean section. Nevertheless, a sensitivity analysis, which included only two studies with balanced groups for age and mode of conception, showed also an increased risk of Caesarean section. This may be partially attributed to the increased risk of fetal

malpresentation and placental malposition in patients with adenomyosis, both representing indications for elective Caesarean section. If patients with adenomyosis are also at an increased risk of failed vaginal delivery and secondary Caesarean section is unclear.

An older study reported a PPH prevalence of 17.2% in women who needed a cesarean hysterectomy, which may suggest an increased risk of severe PPH in women with adenomyosis [45]. The current results are in line with the above observation while one study has also reported a higher rate of placental malpresentation that could contribute to the increased blood loss[13].

Adenomyosis is a very heterogenic disorder with varying extent of lesions, ranging from multiple lesions with diffuse myometrial hypertrophy to more discrete focal lesions [46]. It is plausible that the impact of adenomyosis on the reproductive course is not always the same rather than dependent on the degree of the uterus involvement. Concerning the clinical pregnancy rate after ART two studies compared the effects of focal versus diffuse adenomyosis on clinical pregnancy rate [24,36]. The pooled results gave a statistically non-significant OR of 1.36 favoring focal adenomyosis (CI: 0.67-2.75) [47]. In another prospective study, 152 women had MRI prior to in vitro fertilization [34]. The pregnancy rate in the group with maximum junctional zone thickness <10 vs. >10 mm was 63 vs. 14%. Implantation failure rate was 96% in patients with an average JZ thickness >7 mm and a maximal JZ >10 mm, compared with 38% in other patient groups. This study indicates an increase in adverse implantation outcome in relation to the JZ thickness. Unfortunately, our objective to address this issue could not be adequately met due to the different diagnostic criteria for adenomyosis as well as the inadequate characterization and classification of adenomyosis in the single studies. Certain diagnostic criteria for adenomyosis have been proposed[48] and we strongly suggest that these are systematically used in future studies in order to investigate which adenomyosis characteristics, if any, are the most significant for the reproductive course. A recent article proposed that seven items should be assessed when examining and describing a uterus with adenomyosis by ultrasound: presence, location, differentiation (focal/diffuse), appearance (cystic/non-cystic), uterine layer involvement, extent, and size of lesion [49]. A similar MRI-based classification distinguishing between internal adenomyosis, external adenomyosis and structural-related adenomyoma subtypes with a potential relation for therapeutic strategy has been proposed[50].

The frequent coexistence of adenomyosis with other gynecologic disorders such as endometriosis and uterine fibroids is well known [4, 51]. Both endometriosis and uterine fibroids have been however correlated with adverse pregnancy outcomes [52-56]. To examine adenomyosis as an independent risk factor for adverse reproductive and pregnancy outcomes, ideally, controlled studies matched for the existence of endometriosis and uterine fibroids would be appropriate. In our systematic review, this was the case for two studies on fertility outcomes, which reported no difference in the clinical pregnancy rate between groups [25, 32]. Both studies, however, applied the ultra-long downregulation protocol, which might be the reason for identifying no difference. Two other matched studies for endometriosis reported a higher pooled risk of SGA in patients with adenomyosis [15, 33] indicating that adenomyosis is an endometriosis-independent risk factor for SGA. A similar subanalysis for other outcomes was impossible.

Endometriosis and adenomyosis are considered the expression of a series of cumulative genetic and epigenetic incidents [57]. Some of these underlying genetic variants might trigger certain biological mechanisms, which impair the placentation process and lead to the observed pregnancy and obstetrical complications. A number of these mechanisms have been described including activation of local and systemic inflammatory pathways, dysfunctional endometrium, increased myometrial prostaglandin production, altered uterine contractility and defective myometrial spiral artery remodeling [58]. It is currently unclear if these mechanisms would persist even after excision of adenomyosis similar to what has been recently observed in patients with previously excised deep endometriosis [8]. Furthermore, if certain medical therapies prior to implantation could counteract these pathophysiologic mechanisms remains unknown but represents an intriguing possibility. To date only one study [32], in which ultra-long GnRHa downregulation was applied prior to ART, reported the pregnancy outcomes. Patients with adenomyosis still showed a significantly higher risk of IUGR.

Strengths and limitations

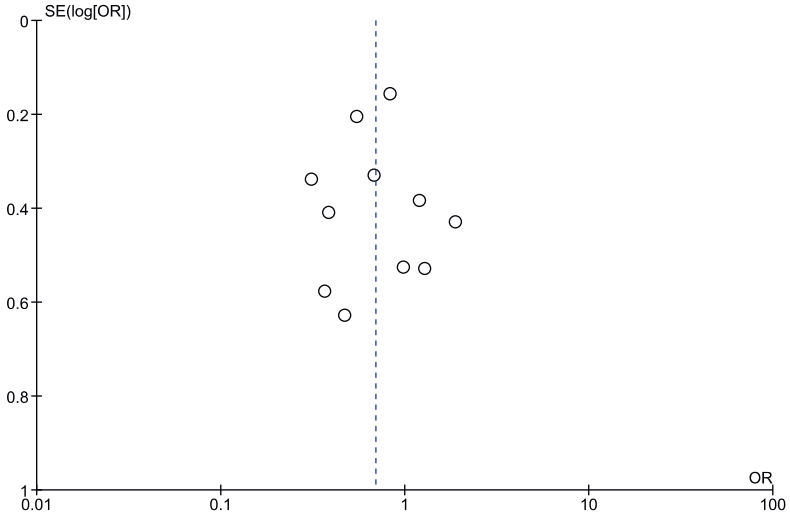
To avoid major bias in data gathering the literature review and data extraction was performed by two investigators in this meta-analysis. Moreover, study heterogeneity was addressed by sensitivity analysis, when possible, excluding studies with unbalanced groups for potential confounders such as age, mode of conception and stimulation protocol for ART. Despite these efforts, study heterogeneity and the intrinsic limitations of controlled observational studies represent sources of potential bias in this study. Such limitations include the diagnostic accuracy of the non-invasive imaging techniques for adenomyosis while the diagnosis would be ideally performed by histology as well as the lack of exclusion of certain pathologies such as peritoneal endometriosis or uterine fibroids.

Conclusion

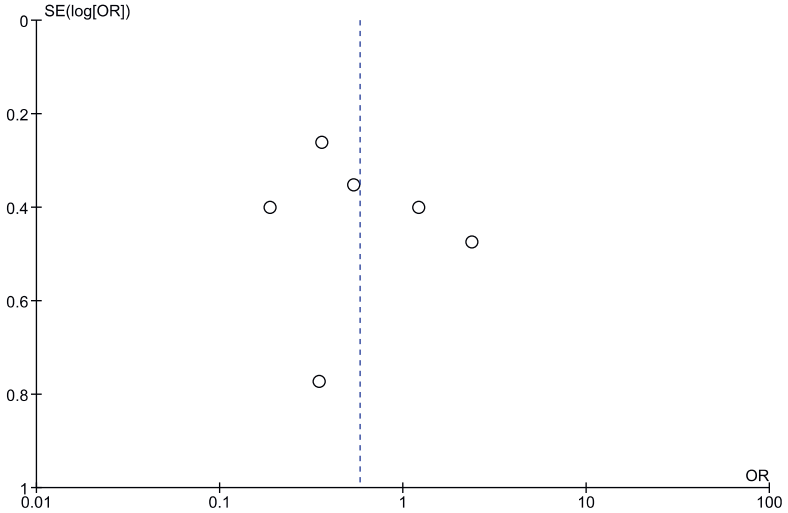
We present a negative association between adenomyosis and fertility outcome especially after short protocol downregulation in ART. This association is less significant or absent in ART with mixed or ultra-long GnRHa protocols but the results are not sufficient for a conclusive evaluation of the most proper ART protocol. Ongoing randomized controlled studies intend to provide reliable conclusive data on the possible protective role of ultra-long GnRHa downregulation in adenomyosis. Adenomyosis also correlates with adverse pregnancy outcomes such as preterm delivery, preeclampsia, Caesarean section, fetal malpresentation, SGA, low birth weight, and PPH. Gynecologists should be aware of these risks to indicate proper pregnancy controls enabling an early diagnosis and treatment of pregnancy complications. Matched controlled studies with proper adenomyosis classification extending from fertility desire to postpartum period are needed to investigate the role of specific adenomyosis subtypes and their treatment in every aspect of the reproductive course.

Supplementary figure

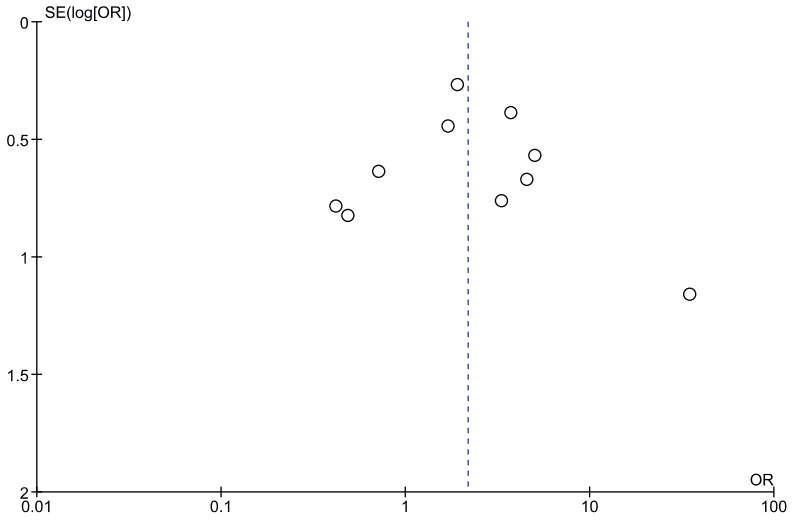
A. Clinical pregnancy rate



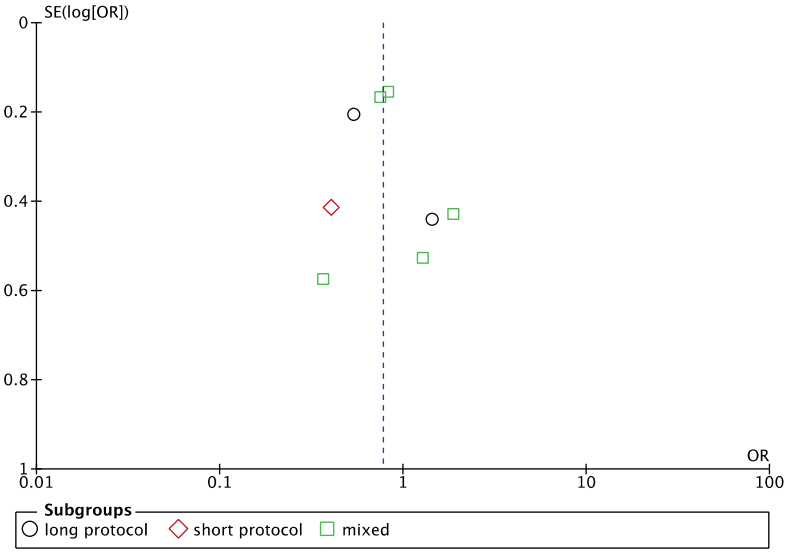
B. Live birth rate



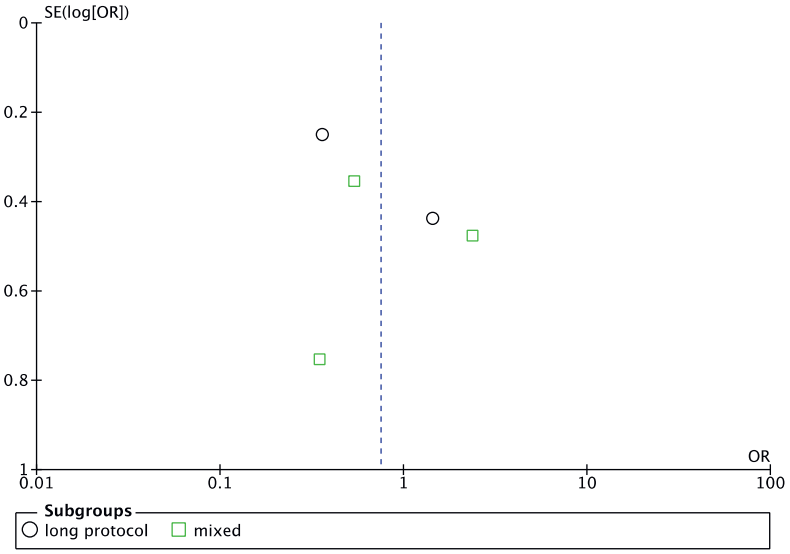
C. Miscarriage rate



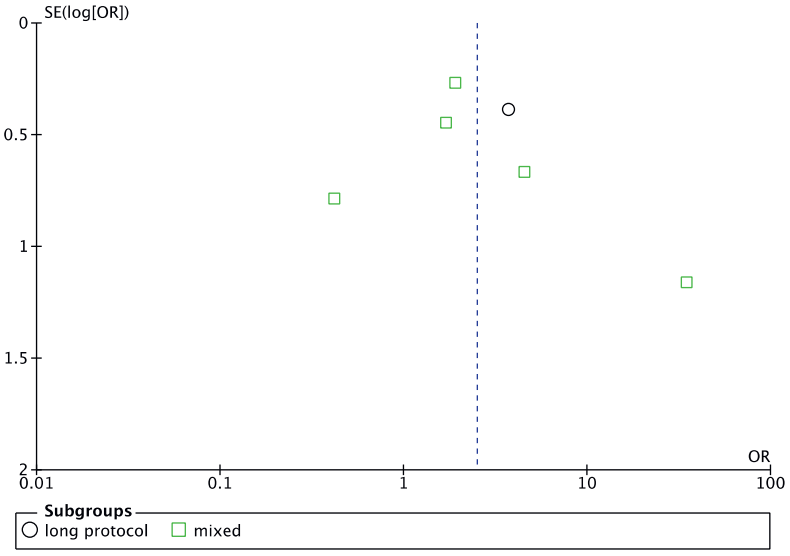
D. Clinical pregnancy rate (age-adjusted)



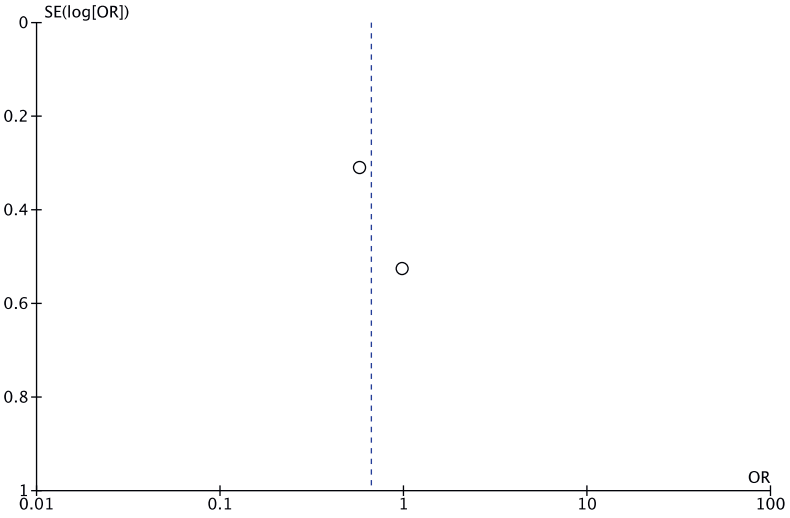
E. Live birth rate (age-adjusted)



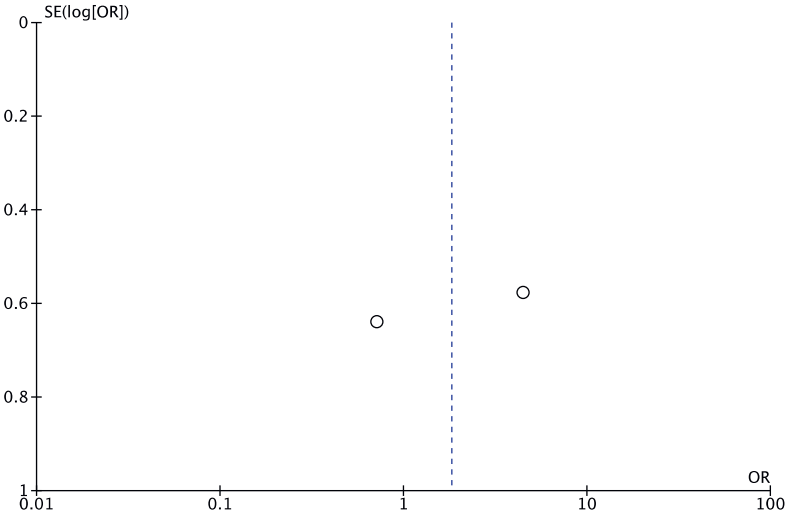
F. Miscarriage rate (age-adjusted)



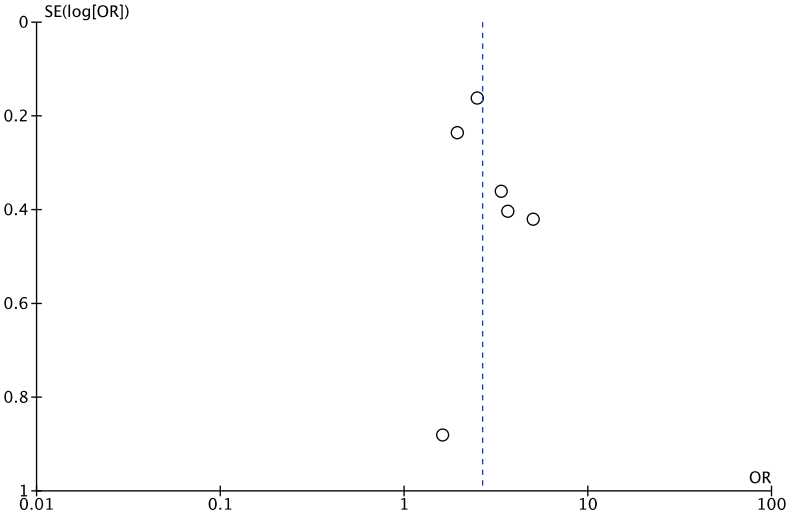
G. Clinical pregnancy rate (endometriosis-adjusted)



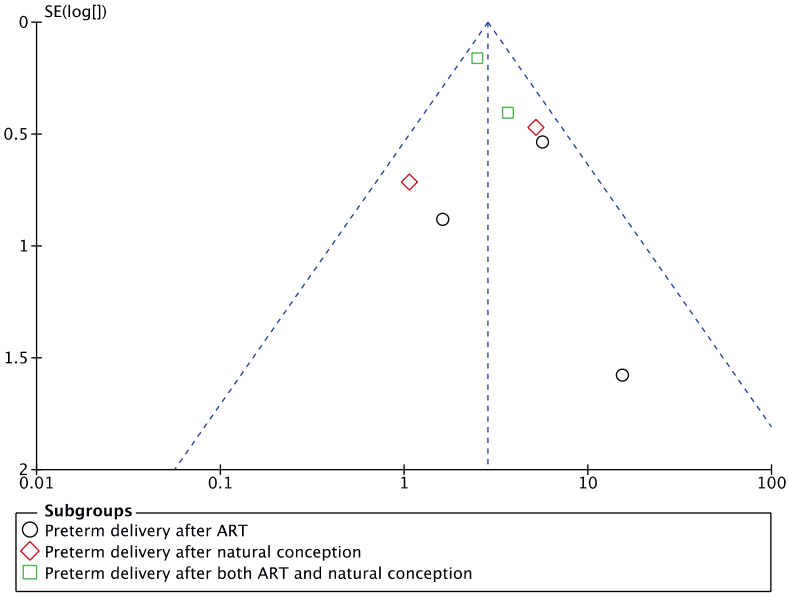
H. Miscarriage rate (endometriosis-adjusted)



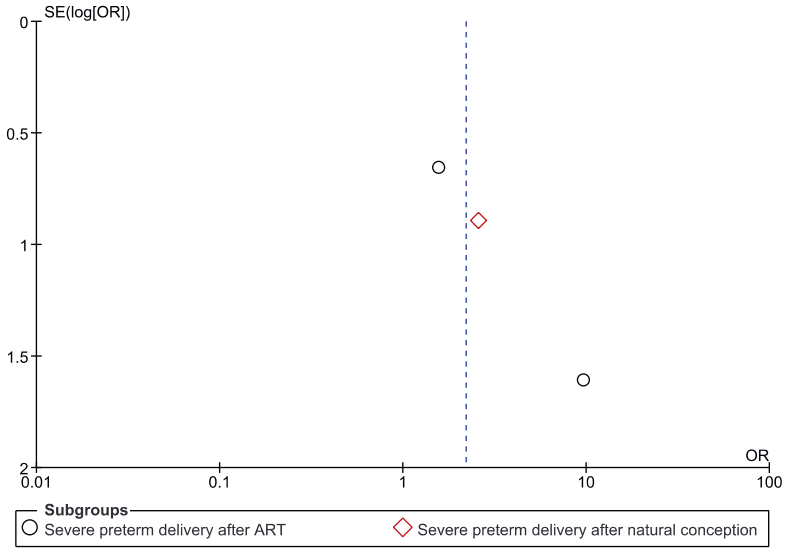
I. Preterm delivery (age-adjusted)



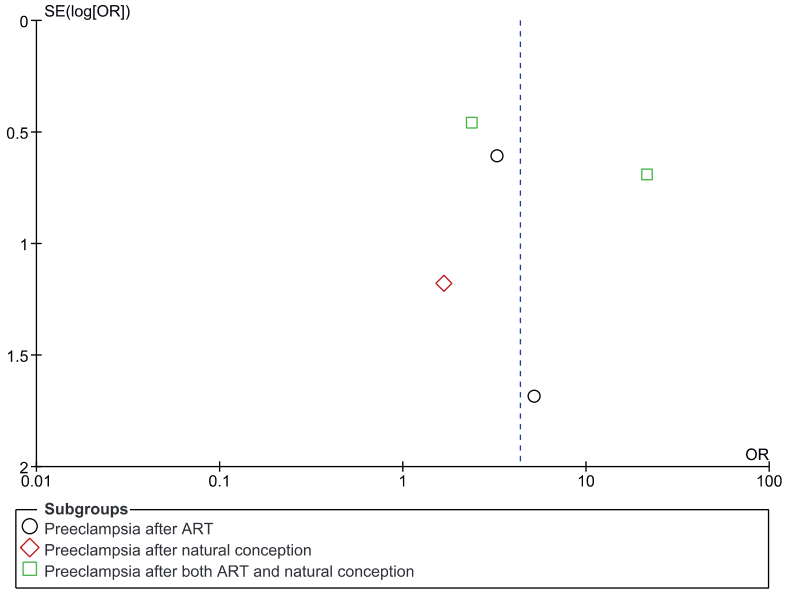
J. Preterm delivery (age- and mode of conception- adjusted)



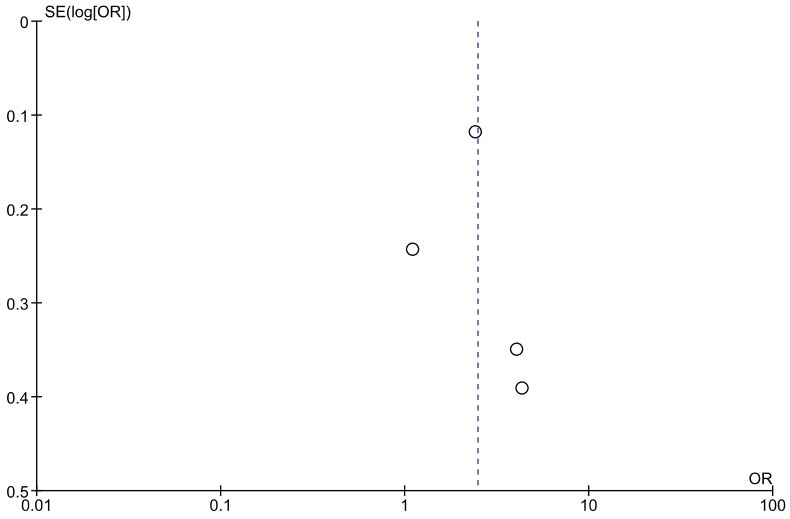
K. Severe preterm delivery



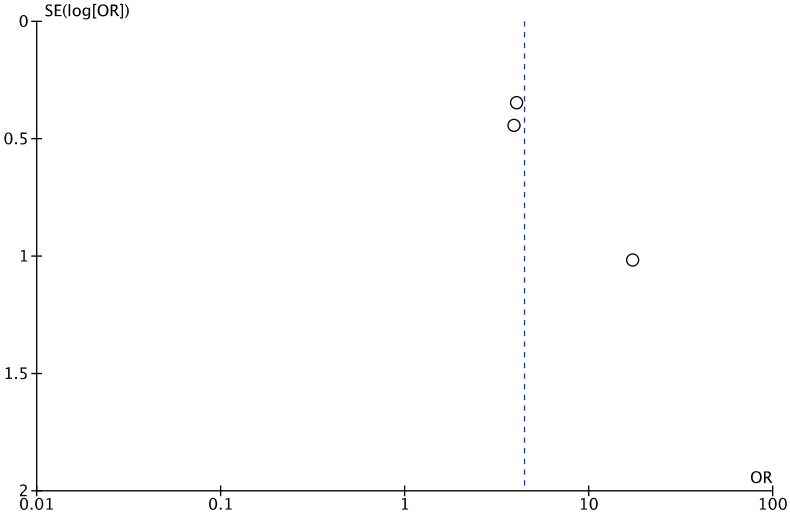
L. Preeclampsia



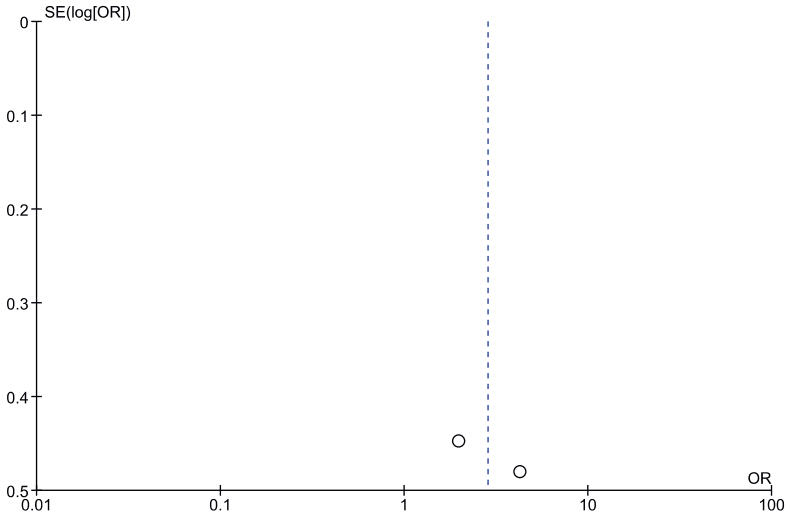
M. C-Section



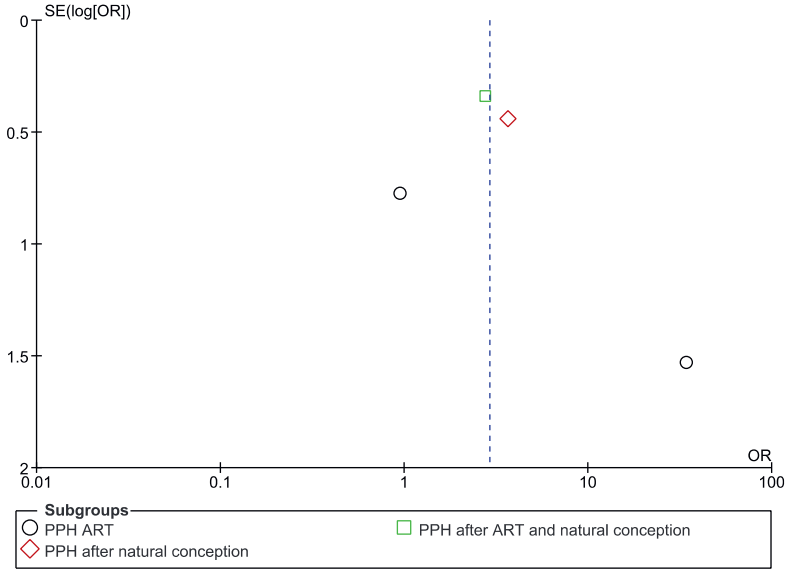
N. C-Section (matched for age and rate of ART)



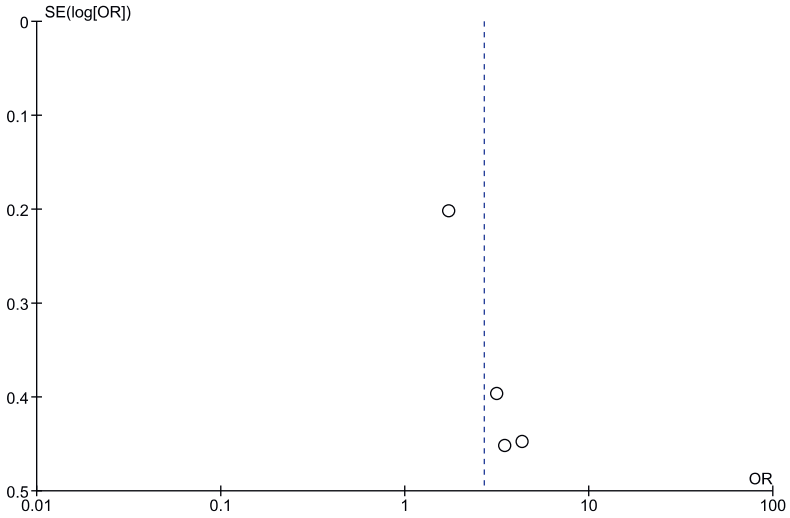
O. Fetal malpresentation



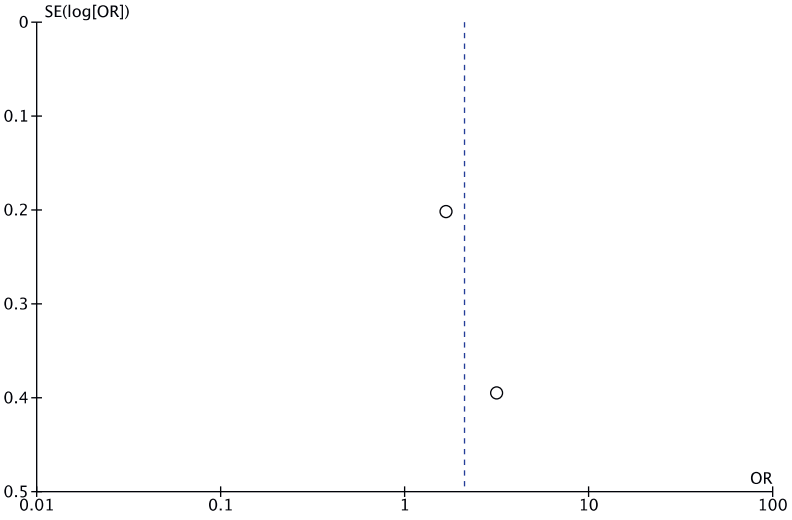
P. Postpartum hemorrhage



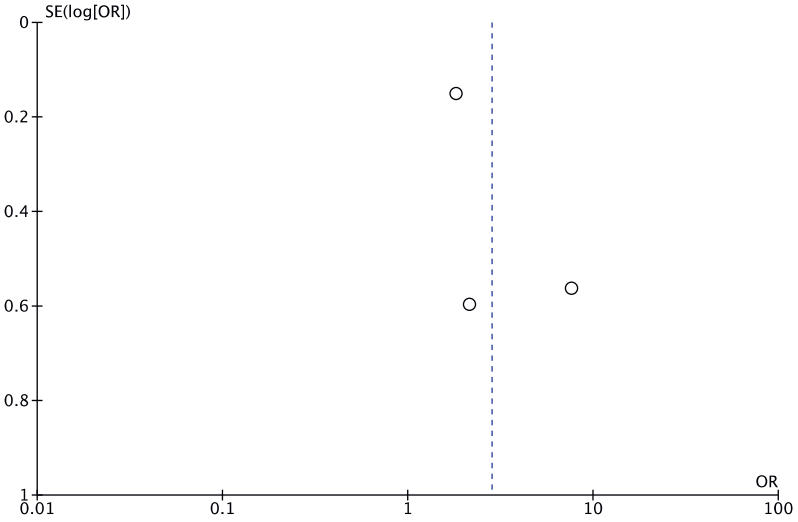
Q. SGA



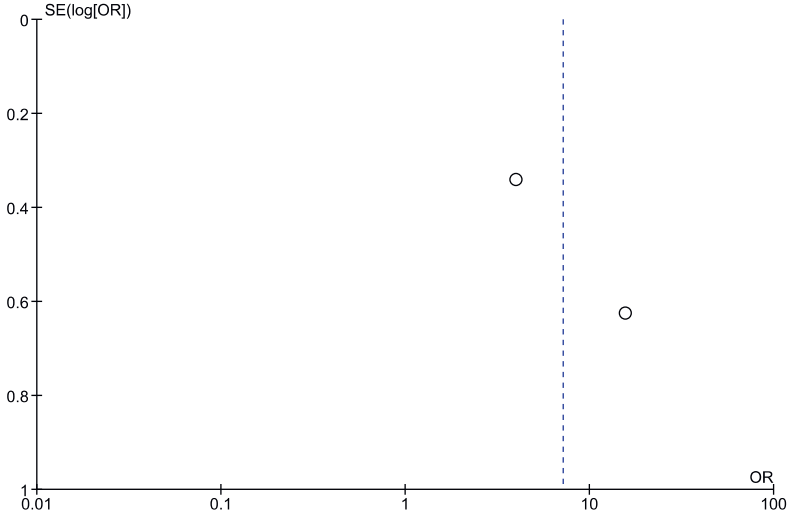
R. SGA (sensitivity analysis endometriosis)



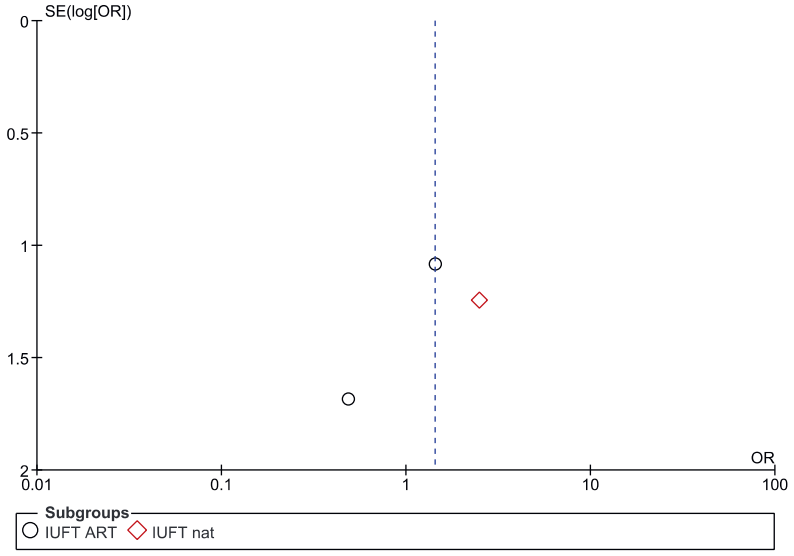
S. Birth weight < 2500g



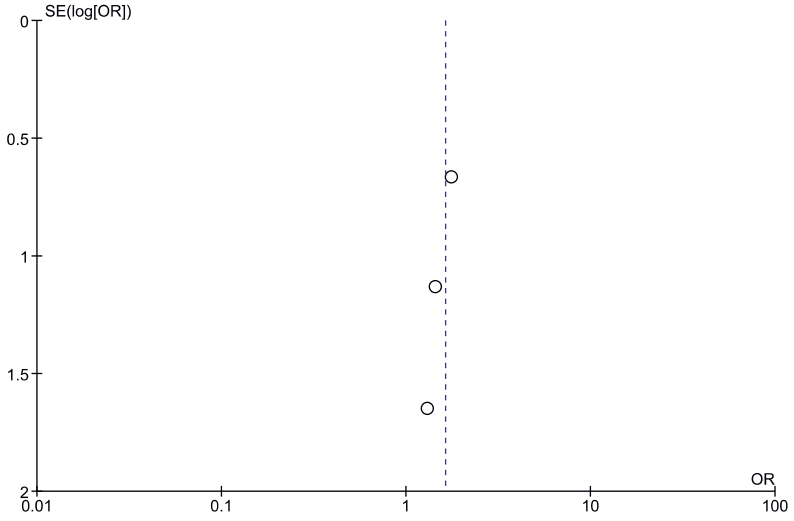
T. Birth weight < 1500g



U. Intrauterine fetal death



V. APGAR



References

1. Abbott JA. Adenomyosis and Abnormal Uterine Bleeding (AUB-A)-Pathogenesis, diagnosis, and management. *Best practice & research Clinical obstetrics & gynaecology* 2017, 40: 68-81.

2. Vercellini P, Vigano P, Somigliana E, Daguati R, Abbiati A, Fedele L. Adenomyosis: epidemiological factors. *Best practice & research Clinical obstetrics & gynaecology* 2006, 20: 465-77.

3. Chapron C, Vannuccini S, Santulli P, Abrao MS, Carmona F, Fraser IS et al. Diagnosing adenomyosis: an integrated clinical and imaging approach. *Human reproduction update* 2020.

4. Lazzeri L, Di Giovanni A, Exacoustos C, Tosti C, Pinzauti S, Malzoni M et al. Preoperative and Postoperative Clinical and Transvaginal Ultrasound Findings of Adenomyosis in Patients With Deep Infiltrating Endometriosis. *Reproductive sciences* 2014, 21: 1027-33.

5. Lalani S, Choudhry AJ, Firth B, Bacal V, Walker M, Wen SW et al. Endometriosis and adverse maternal, fetal and neonatal outcomes, a systematic review and meta-analysis. *Human reproduction* 2018, 33: 1854-65.

6. Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A, Giorgione V et al. A systematic review on endometriosis during pregnancy: diagnosis, misdiagnosis, complications and outcomes. *Human reproduction update* 2016, 22: 70-103.

7. Leone Roberti Maggiore U, Inversetti A, Schimberni M, Viganò P, Giorgione V, Candiani M. Obstetrical complications of endometriosis, particularly deep endometriosis. *Fertility and sterility* 2017, 108: 895-912.

8. Nirgianakis K, Gasparri ML, Radan AP, Villiger A, McKinnon B, Mosimann B et al. Obstetric complications after laparoscopic excision of posterior deep infiltrating endometriosis: a case-control study. *Fertility and sterility* 2018, 110: 459-66.

9. Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. *Human reproduction update* 2019, 25: 592-632.

10. Qin JB, Sheng XQ, Wu D, Gao SY, You YP, Yang TB et al. Worldwide prevalence of adverse pregnancy outcomes among singleton pregnancies after in vitro fertilization/intracytoplasmic sperm injection: a systematic review and meta-analysis. *Archives of gynecology and obstetrics* 2017, 295: 285-301.

11. Vercellini P, Consonni D, Dridi D, Bracco B, Frattaruolo MP, Somigliana E. Uterine adenomyosis and in vitro fertilization outcome: a systematic review and meta-analysis. *Human reproduction* 2014, 29: 964-77.

12. Mochimaru A, Aoki S, Oba MS, Kurasawa K, Takahashi T, Hirahara F. Adverse pregnancy outcomes associated with adenomyosis with uterine enlargement. *The journal of obstetrics and gynaecology research* 2015, 41: 529-33.

13. Hashimoto A, Iriyama T, Sayama S, Nakayama T, Komatsu A, Miyauchi A et al. Adenomyosis and adverse perinatal outcomes: increased risk of second trimester miscarriage, preeclampsia, and placental malposition. The journal of maternal-fetal & neonatal medicine : *the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* 2018, 31: 364-9.

14. Juang CM, Chou P, Yen MS, Twu NF, Horng HC, Hsu WL. Adenomyosis and risk of preterm delivery. *BJOG : an international journal of obstetrics and gynaecology* 2007, 114: 165-9.

15. Scala C, Leone Roberti Maggiore U, Racca A, Barra F, Vellone VG, Venturini PL et al. Influence of adenomyosis on pregnancy and perinatal outcomes in women with endometriosis. *Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2018, 52: 666-71.

16. Hayden JA, Cote P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. *Annals of internal medicine* 2006, 144: 427-37.

17. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986, 7: 177-88.

18. Egger M DSG, Altman D. Systematic reviews in health care: meta-analysis in context. *London: BMJ Publishing Group*, 2001.

19. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003, 327: 557-60.

20. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009, 339: b2535.

21. Chiang CH, Chang MY, Shiau CS, Hou HC, Hsieh TT, Soong YK. Effect of a sonographically diffusely enlarged uterus without distinct uterine masses on the outcome of in vitro fertilization-embryo transfer. *Journal of assisted reproduction and genetics* 1999, 16: 369-72.

22. Salim R, Riris S, Saab W, Abramov B, Khadum I, Serhal P. Adenomyosis reduces pregnancy rates in infertile women undergoing IVF. *Reproductive biomedicine online* 2012, 25: 273-7.

23. Ballester M, d'Argent EM, Morcel K, Belaisch-Allart J, Nisolle M, Daraï E. Cumulative pregnancy rate after ICSI–IVF in patients with colorectal endometriosis: results of a multicentre study. *Human reproduction* 2012, 27: 1043-9.

24. Benaglia L, Cardellicchio L, Leonardi M, Faulisi S, Vercellini P, Paffoni A et al. Asymptomatic adenomyosis and embryo implantation in IVF cycles. *Reproductive biomedicine online* 2014, 29: 606-11.

25. Mijatovic V, Florijn E, Halim N, Schats R, Hompes P. Adenomyosis has no adverse effects on IVF/ICSI outcomes in women with endometriosis treated with long-term pituitary down-regulation before IVF/ICSI. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2010, 151: 62-5.

26. Costello MF, Lindsay K, McNally G. The effect of adenomyosis on in vitro fertilisation and intra-cytoplasmic sperm injection treatment outcome. *European Journal of Obstetrics & Gynecology and Reproductive Biology* 2011, 158: 229-34.

27. Youm HS, Choi YS, Han HD. In vitro fertilization and embryo transfer outcomes in relation to myometrial thickness. *Journal of assisted reproduction and genetics* 2011, 28: 1135-40.

28. Martínez-Conejero JA, Morgan M, Montesinos M, Fortuño S, Meseguer M, Simón C et al. Adenomyosis does not affect implantation, but is associated with miscarriage in patients undergoing oocyte donation. *Fertility and sterility* 2011, 96: 943-50.e1.

29. Thalluri V, Tremellen KP. Ultrasound diagnosed adenomyosis has a negative impact on successful implantation following GnRH antagonist IVF treatment. *Human reproduction* 2012, 27: 3487-92.

30. Yan L, Yan L, Ding L, Tang R, Chen ZJ. Effect of Adenomyosis on In Vitro Fertilization/Intracytoplasmic Sperm Injection Outcomes in Infertile Women: A Retrospective Cohort Study. *Gynecologic and obstetric investigation* 2014, 77: 14-8.

31. Shin YJ, Kwak DW, Chung JH, Kim MY, Lee SW, Han YJ. The Risk of Preterm Births Among Pregnant Women With Adenomyosis. *Journal of ultrasound in medicine* 2018, 37: 1937-43.

32. Sharma S, Bathwal S, Agarwal N, Chattopadhyay R, Saha I, Chakravarty B. Does presence of adenomyosis affect reproductive outcome in IVF cycles? A retrospective analysis of 973 patients. *Reproductive biomedicine online* 2019, 38: 13-21.

33. Yamaguchi A, Kyozuka H, Fujimori K, Hosoya M, Yasumura S, Yokoyama T et al. Risk of preterm birth, low birthweight and small-for-gestational-age infants in pregnancies with adenomyosis: A cohort study of the Japan Environment and Children's Study. *Acta obstetricia et gynecologica Scandinavica* 2019, 98: 359-64.

34. Maubon A, Faury A, Kapella M, Pouquet M, Piver P. Uterine junctional zone at magnetic resonance imaging: a predictor of in vitro fertilization implantation failure. *The journal of obstetrics and gynaecology research* 2010, 36: 611-8.

35. Imaoka I, Ascher SM, Sugimura K, Takahashi K, Li H, Cuomo F et al. MR imaging of diffuse adenomyosis changes after GnRH analog therapy. *Journal of magnetic resonance imaging : JMRI* 2002, 15: 285-90.

36. Park CW, Choi MH, Yang KM, Song IO. Pregnancy rate in women with adenomyosis undergoing fresh or frozen embryo transfer cycles following gonadotropin-releasing hormone agonist treatment. *Clinical and experimental reproductive medicine* 2016, 43: 169-73.

37. Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. *Gynecological Endocrinology* 2013, 29: 1026-30.

38. Surrey ES. To suppress or not to suppress? If that is the question, has it been answered? *Fertility and sterility* 2020, 113: 763-4.

39. Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ et al. Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet* 2019, 393: 1310-8.

40. Younes G, Tulandi T. Effects of adenomyosis on in vitro fertilization treatment outcomes: a meta-analysis. *Fertility and sterility* 2017, 108: 483-90.e3.

41. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Research synthesis methods* 2010, 1: 97-111.

42. Vercellini P, Consonni D, Barbara G, Buggio L, Frattaruolo MP, Somigliana E. Adenomyosis and reproductive performance after surgery for rectovaginal and colorectal endometriosis: a systematic review and meta-analysis. *Reproductive biomedicine online* 2014, 28: 704-13.

43. Barrier BF, Malinowski MJ, Dick EJ, Hubbard GB, Bates GW. Adenomyosis in the baboon is associated with primary infertility. *Fertility and sterility* 2004, 82: 1091-4.

44. Pandey S, Shetty A, Hamilton M, Bhattacharya S, Maheshwari A. Obstetric and perinatal outcomes in singleton pregnancies resulting from IVF/ICSI: a systematic review and meta-analysis. *Human reproduction update* 2012, 18: 485-503.

45. Sandberg EC, Cohn F. Adenomyosis in the gravid uterus at term. *American journal of obstetrics and gynecology* 1962, 84: 1457-65.

46. Chapron C, Tosti C, Marcellin L, Bourdon M, Lafay-Pillet MC, Millischer AE et al. Relationship between the magnetic resonance imaging appearance of adenomyosis and endometriosis phenotypes. *Human reproduction* 2017, 32: 1393-401.

47. Younes G, Tulandi T. Effects of adenomyosis on in vitro fertilization treatment outcomes: a meta-analysis. *Fertility and sterility* 2017, 108: 483-90 e3.

48. Gordts S, Grimbizis G, Campo R. Symptoms and classification of uterine adenomyosis, including the place of hysteroscopy in diagnosis. *Fertility and sterility* 2018, 109: 380-8 e1.

49. Van den Bosch T, de Bruijn AM, de Leeuw RA, Dueholm M, Exacoustos C, Valentin L et al. Sonographic classification and reporting system for diagnosing adenomyosis. *Ultrasound in obstetrics & gynecology* 2019, 53: 576-82.

50. Bazot M, Darai E. Role of transvaginal sonography and magnetic resonance imaging in the diagnosis of uterine adenomyosis. *Fertility and sterility* 2018, 109: 389-97.

51. Eisenberg VH, Arbib N, Schiff E, Goldenberg M, Seidman DS, Soriano D. Sonographic Signs of Adenomyosis Are Prevalent in Women Undergoing Surgery for Endometriosis and May Suggest a Higher Risk of Infertility. *BioMed research international* 2017, 2017: 8967803.

52. Zullo F, Spagnolo E, Saccone G, Acunzo M, Xodo S, Ceccaroni M et al. Endometriosis and obstetrics complications: a systematic review and meta-analysis. *Fertility and sterility* 2017, 108: 667-72.e5.

53. Karlsen K, Schioler Kesmodel U, Mogensen O, Humaidan P, Ravn P. Relationship between a uterine fibroid diagnosis and the risk of adverse obstetrical outcomes: a cohort study. *BMJ open* 2020, 10: e032104.

54. Klatsky PC, Tran ND, Caughey AB, Fujimoto VY. Fibroids and reproductive outcomes: a systematic literature review from conception to delivery. *American journal of obstetrics and gynecology* 2008, 198: 357-66.

55. Lam S-J, Best S, Kumar S. The impact of fibroid characteristics on pregnancy outcome. *American journal of obstetrics and gynecology* 2014, 211: 395.e1-.e5.

56. Gasparri ML, Nirgianakis K, Taghavi K, Papadia A, Mueller MD. Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis. *Archives of gynecology and obstetrics* 2018, 298: 27-34.

57. Koninckx PR, Zupi E, Martin DC. Endometriosis and pregnancy outcome. *Fertility and sterility* 2018;110:406-7.

58. Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. *Human reproduction update* 2016, 22: 104-15.

Chapter 8
General discussion

Discussion

Endometriosis and adenomyosis represent two highly significant diseases based on their high incidence in premenopausal women and related disease burden, which involves chronic pain, uterine bleeding disorders and infertility. The chronic nature of endometriosis with frequent recurrences after surgery, the common coexistence with adenomyosis and the limitations of current medical treatments constitute serious challenges of managing these disorders. In addition, the effect on the reproductive course constitute important, yet relatively unexplored aspects of these diseases.

In chapter 2, we examined the recurrence potential of the different subtypes of endometriosis (superficial peritoneal lesions (SUP), ovarian endometriomas (OMA) and deep infiltrating endometriosis (DIE)) after surgery. We detected no difference in the recurrence potential but a high percentage of patients presented with more severe lesions subtypes, particularly DIE at recurrence. This trend towards more severe endometriosis subtypes suggests disease progression, which may occur overtime irrespective of surgical removal.

Whether endometriosis represents a progressive disease that worsens over time attracts much attention. A previous 3-year prospective study suggested that endometriosis is a progressive disease [1] which was supported by a review of adolescence endometriosis [2], with an additional study showing development from peritoneal to ovarian endometriosis, including uterosacral ligament lesions during a 2-5 years follow up [3]. On the contrary, an analysis of randomized control studies (RCT) in adolescents showed 71% of the patients without endometriosis excision did not progress [4]. However, a single RCT contributed the majority of cases to this analysis with a short follow up of only 4 to 6 months [5]. The findings of our study agree with the suggestion of progression and extend them to a broader population with a longer follow up.

However, since endometriosis is a highly heterogenic disease with different lesion subtypes and disease burden, it is plausible that the recurrence potential as well as the disease progression may differ significantly among individuals. This explains why some patients present with severe DIE already in adolescence while others present DIE at later ages only. A number of factors could be crucial for these differences: affected females genotype, exposures to environmental factors (e.g. toxins, pathogens, chemicals, pollution, stress, diet), type of surgical treatment, type and duration of medical treatment.

In chapter 3, we aimed to identify such clinical risk factors for recurrence after laparoscopic segmental bowel resection for endometriosis. Positive bowel resection margins as well as age < 31 years and BMI ≥ 23 kg/m^2 appeared to be independent risk factors for disease recurrence. This was the first study to show a correlation between the histopathological margins collected from the resected tissue and disease recurrence.

Two previous studies have reported no correlation [6, 7]; however, the first study reported that in 66 out of 92 patients a disc resection was performed, which in this case cannot be compared with our study population in which disc resections were excluded. In the second study, there was a different outcome measured since only

the recurrence of pain but not the subsequent re-operation for endometriosis was examined. More importantly in this study, the follow up time was limited to a median of 18 months after surgery, whereas most recurrences in our study took place only between 17 and 90 months after surgery.

An association between positive resection margins and disease recurrence is not equal with causality. Whether the endometriotic lesion that precipitates the need for a repeat operation is derived from such residual lesions or a new endometriotic lesion is not clear. It may be tempting to speculate that positive bowel resection margins imply an overall more advanced state of disease and thus is indicative of a higher risk of recurrence. Indeed, it was previously shown that in patients presenting with bowel endometriosis, microscopically complete excision of bowel endometriosis may be impossible because of bowel occult microscopic endometriosis implants located far from macroscopic nodules [8, 9], while no association with pelvic or digestive symptoms after 1-year follow-up was identified [10]. Moreover, in patients managed via low rectal resections, the preoperative digestive symptoms due to endometriosis may be in turn replaced by more unpleasant postoperative digestive functional disorders due to rectum resection syndrome [11]. For these reasons, the performance of large bowel resections with the goal of reducing the incidence of positive resection margins and the recurrence risk cannot be supported by the available clinical data.

The finding that younger patients have a higher risk of recurrence is not surprising and has already been presented by other authors [12]. Moreover, our study, which shows that high BMI is a predictive factor for recurrence, is in line with the study from Netzhat et al. [13]. Many epidemiological studies have identified other risk factors for recurrence although the results are sometimes conflicting [14]. This could be secondary to the definition of recurrence (recurrence of pain or recurrence of endometriosis lesions), the patient population, the length of follow-up and the difference in surgical procedures.

It should be underlined that for all patients in our study a complete endometriosis excision was performed. The incompleteness of the surgical excision has been previously recognized as a risk factor for recurrence [15] although it may be more proper to define it as endometriosis persistence rather than recurrence. Therefore, the performance of such complex surgeries from expert and dedicated teams in endometriosis is advocated [16].

A limitation of our study is that no information on the postoperative medication could be obtained so that the influence of medical treatment on endometriosis recurrence could not be evaluated. However, the data seems to be clear regarding the effect of postoperative medical treatment on disease recurrence [17] so that currently adjuvant hormonal therapy is advised to all patients after surgery to avoid disease recurrence [18, 19]. However, some patients may intrinsically have a much lower risk of recurrence than others, while others may be resistant to medical treatment. Adjuvant medical tretament in these patients is not useful and may cause unnecessary side effects and an increase in health care costs. The identification of high-risk patients who may benefit the most from drug intervention remains a challenge.

Endometriosis is an estrogen-dependent disease with a significant inflammatory aspect. Cytokines act as both paracrine and autocrine signaling molecules that modulate a variety of cellular functions including proliferation, survival, and differentiation that contributes to the progression of endometriosis [20]. They may also play a role in stimulating peripheral nerve sensitization [21] and contribute to the chronic pelvic pain experienced by patients [22]. In chapter 4, we sought to evaluate the effect of GnRHa, an important drug for endometriosis, on the inflammation of the peritoneal microenvironment. We achieved this by analyzing several biomarkers in the peritoneal fluid, which have been previously found to be elevated in women with endometriosis. We observed a significant regression of four biomarkers (IL-8, PAPP-A, glycodelin-A and midkine) in patients who had been treated with GnRHa prior to endometriosis surgery. This implies a GnRHa-mediated significant regression in the inflammatory nature of the peritoneal microenvironment, which might partially explain the effect on the endometriosis-associated pain.

A similar effect of dienogest (DNG), another first-line drug for endometriosis, on midkine was identified in a more recent study of our team [23]. Both GnRHa and progestins such as DNG are believed to reduce the endometriosis pain through the activation of pituitary progesterone receptors (PR) creating a hypo-estrogenic systemic environment and amenorrhea. This systemic influence however may also be accompanied by significant, unwanted side effects such as hot flushes, sleep disorders, decreased libido and mood disorders. Understanding the influence on inflammation of these drugs and how this is mediated may provide the opportunity to enhance their local effects and minimize their unwanted systemic effects, while retaining or improving their clinical efficacy. Indeed, in an in-vitro study we identified that DNG mediated a suppression of cytokine mRNA production and cytokine secretion [24]. Furthermore, the TNF-α-stimulated proliferation of endometrial stroma cells was suppressed by DNG (Figure 1).

These results suggest that DNG has a direct effect on endometrial lesions in addition to their systemic effects that may interrupt endometriotic lesion progression. Although it is not clear how progestins suppress the level of cytokine secretion, numerous mechanisms are possible and should be considered in future research projects (Figure 2). This could facilitate the development of novel therapeutics with improved clinical profile that would emphasize non-hormonal functions of GnRHa and progestins.

Furthermore, the impact of GnRHa on intraperitoneal cytokine concentrations might explain the increased clinical pregnancy rate through administering GnRHa for three to six months prior to IVF or ICSI in women with endometriosis [25]. This effect seems to be more obvious in patients with severe stages of endometriosis [26, 27]. Further studies are needed to estimate if GnRHa prior to conception could also reduce the increased rate of pregnancy complications, which is observed in women with endometriosis.

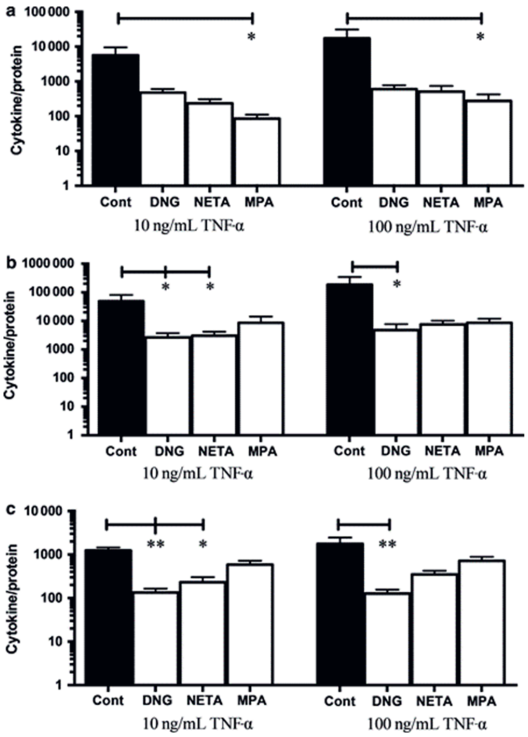


Figure 1. Inhibition of TNF-α-stimulated cytokine secretion in the presence of progestins (adopted from Grandi et al[24]) (a) IL-6, (b) IL-8, and (c) MCP-1 at TNF-α 10 ng/mL and TNF-α 100 ng/mL. *P<.05, **P<.01

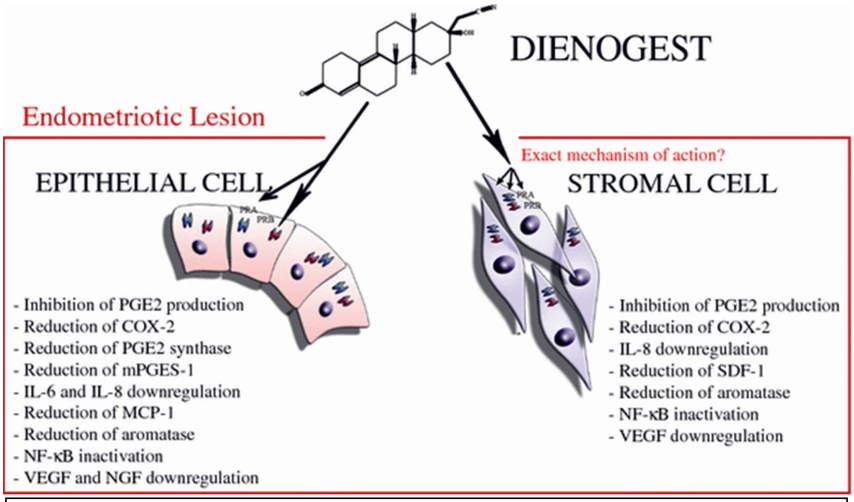


Figure 2. DNG influence on the inflammatory response (adopted from Grandi et al[84])

In chapter 5, the risk of placenta previa after assisted reproductive technology (ART) was found threefold higher in patients with than without endometriosis and this difference was statistically significant. This finding suggests that endometriosis is a risk factor for placental disorders independently of the way of conception since all included patients in this study had conceived only after ART.

A recent meta-analysis had also identified a higher risk of placental and other obstetrical complications in patients with endometriosis [28]. However, in this study the influence of the way of conception had not been taken into account. This is important since ART itself has been associated with an increased risk of pregnancy complications and found to be associated with a sixfold increased risk of placenta previa [29-31]. The American College of Obstetricians and Gynecologists (ACOG) recently released recommendations on the management of these risks [32]. With our work, we have conclusively shown that endometriosis is a further additive risk factor for placental complications.

The increased risks of pregnancy and obstetrical outcomes has been previously shown in patients with surgically untreated DIE [33]. However, no study has studied if previous surgical excision has the potential to decrease the risk of pregnancy complications. Moreover, no guidelines exist on the way of delivery in patients previously operated for severe DIE with colorectal infiltration. In chapter 6, we aimed to answer the above questions by performing a matched case-control study. Patients with previously excised posterior DIE, similarly to women with endometriosis in general, were found to have a statistically significant increased risk of placenta previa, gestational hypertension, and IUGR compared with women without endometriosis. Moreover, the possibility of successful vaginal birth, if attempted, was high and similar to that in the control group. Except for a higher postpartal blood loss in the endometriosis group, all other delivery and neonatal risks were similar between groups.

The strengths of this study include the clear inclusion criteria of patients only with completely excised severe posterior DIE, with high incidence of bowel surgery and partial resection of posterior vaginal fornix, as well as the comparison with a controlled group matched for the most important confounding outcomes.

That the rate of pregnancy complications after surgical removal of endometriosis remains uninfluenced can be easily explained when the pathophysiology of pregnancy complications in patients with endometriosis is considered (Figure 3). Previous surgery for DIE is unlikely to treat these abnormalities since they represent mainly a preexisting predisposition rather than a consequence of DIE lesion presence. Similarly, however, our study suggests that no additional adverse effects not already related to the presence of endometriosis itself are created through a previous surgery for endometriosis.

Finally, concerns that the surgery of the rectovaginal septum with or without bowel or vaginal involvement may predispose to failed vaginal delivery are refuted by this study. Women trying to deliver vaginally succeed with a similar rate as endometriosis-free women, although further studies with increased numbers of

patients delivering vaginally are required to conclusively determine the safety of vaginal delivery in this specific group of patients.

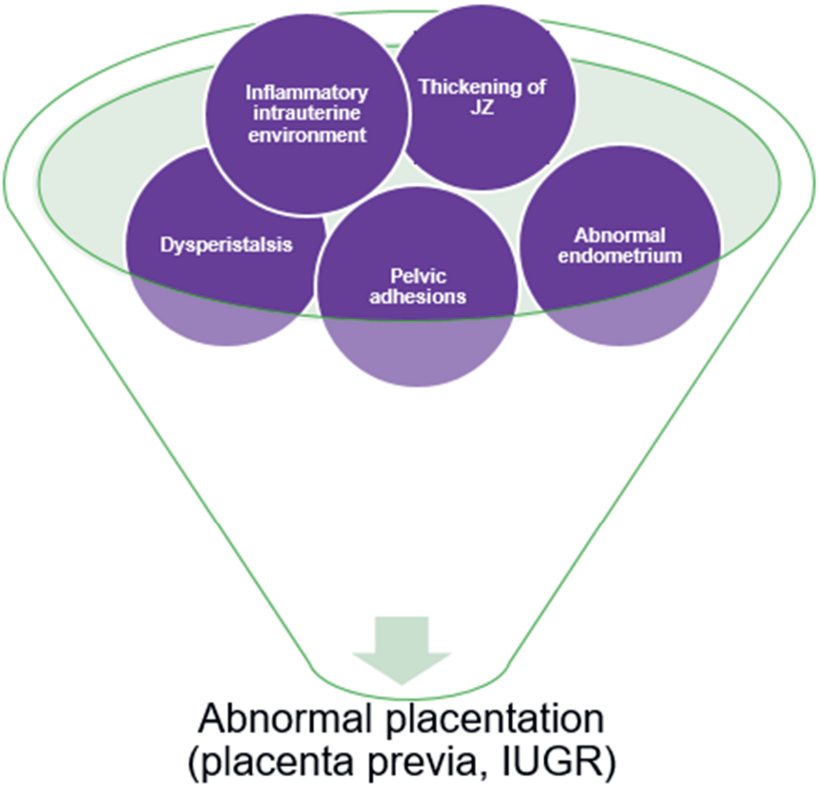


Figure 3. Possible factors associated to endometriosis and adenomyosis that lead to abnormal placentation and adverse pregnancy outcomes
JZ: junctional zone, IUGR: intrauterine growth restriction

As shown above, it has recently emerged that endometriosis may negatively influence pregnancy and neonatal outcomes. The negative correlation remains even after previously excised endometriosis (Chapter 6), which suggests that other mechanisms are involved (Figure 3). One explanation could be that the frequently coexistent adenomyosis, which remains after endometriosis surgery, is likely to cause an abnormal placentation leading to adverse pregnancy outcomes.

The current literature includes a number of studies investigating the fertility and pregnancy outcome in patients with adenomyosis. By performing a systematic review and meta-analysis in chapter 7 we found that adenomyosis is associated with a significantly lower clinical pregnancy rate and higher miscarriage rate after ART, especially when a short GnRH agonist or antagonist protocol is administered for ovarian stimulation. Moreover, adenomyosis is associated with a higher risk of preterm delivery, preeclampsia, cesarean section, fetal malpresentation, low birth weight, and PPH. The association could be confirmed after adjustment of these outcomes for age and mode of conception.

We performed several sensitivity analyses in order to eliminate the confounders such as mode of conception, age and coexistence of endometriosis as well as to examine the impact of different adenomyosis subtypes. Unfortunately, our objective to address the latter could not be adequately met due to the different diagnostic criteria for adenomyosis as well as the inadequate characterization and classification of adenomyosis in the single studies.

A very interesting finding is the possible positive effect of the ultra-long GnRHa pretreatment before ART on the fertility outcome. Ultra-long GnRHa protocols produce a period of estrogen deficiency that may temporarily inactivate adenomyosis, reduce the uterine volume and normalize some of the distorted endometrial functions [34, 35]. This period of potentially therapeutic estrogen deficiency does not occur in GnRH antagonist or short GnRHa cycles. The positive effect on fertility outcomes is further supported by two retrospective controlled studies (RCT) comparing GnRHa pretreatment versus no treatment before fresh–embryo [36] and frozen-embryo transfer [37]. However, these results are not conclusive and currently two RCT protocols are registered in the U.S. National Library of Medicine aiming at elucidating this issue [38, 39].

If the positive effect of prolonged GnRHa prior to ART on fertility outcome is confirmed, concerns about the excessive ovarian suppression, especially in women with reduced ovarian reserve, still need to be addressed. Given recent advances in vitrification technology resulting in enhanced embryo survival and pregnancy rates [27], it could be plausible to firstly vitrify embryos at the blastocyst stage to confirm adequate development and then administer prolonged GnRHa before endometrial preparation with the aim to inactivate adenomyosis, reduce the uterine volume and maybe normalize some of the distorted endometrial functions. This would eliminate concerns regarding excessive ovarian suppression without affecting any possible beneficial impact on fertility outcomes provided by GnRHa. However, one has to keep in mind that frozen blastocyst transfers are associated with a higher risk of preeclampsia (RR 3.13, 95% CI 1.06–9.30, p=0.029) [40]. This risk has to be weighed against the potential benefits of the above approach. Finally, it is currently unknown if alternative treatments with less side effects such as progestins could also be beneficial prior to ART.

Interestingly, the positive effect of the ultra-long GnRHa on fertility outcomes has also been shown in a Cochrane analysis for patients with endometriosis [25]. However, a more recent randomized controlled study failed to show such a benefit for ASRM stage I–II endometriosis patients [26]. Possible explanations are that for these low stage endometriosis patients the inflammatory microenvironment in the follicle and peritoneal fluid is not very profound or that these patients do not suffer from concomitant adenomyosis. On the contrary, for patients with severe endometriosis the coexistence with adenomyosis has been reported to be 40-50% [41, 42]. Therefore, ultra-long GnRHa administration could be beneficial only for severe endometriosis and adenomyosis by reducing the endometriosis-associated inflammation and restoring the adenomyosis-associated uterine function deficiencies.

If other medical therapies such as progestins prior to ART could also positively affect the fertility outcome is currently unknown but represents an intriguing

possibility. Similarly, it is uncertain if specific medical therapies prior to implantation could counteract the pathophysiologic mechanisms involved in the observed higher rate of pregnancy complications in patients with endometriosis and adenomyosis. To date only one study [43], in which ultra-long GnRHa downregulation was applied prior to ART, reported the pregnancy outcomes. Patients with adenomyosis still showed a significantly higher risk of IUGR.

Implications for clinical practice

The high incidence of recurrence after endometriosis surgery indicates the chronic nature of this disease. This seems to be independent of the lesion subtype while it is possible that for some cases there is an evolution to more severe lesion subtypes (deep-infiltrating lesions) over each recurrence [44]. Recurrences are observed even after radical surgeries that include a segmental bowel resection. We identified certain risk factors for recurrence such as young age and positive bowel resection margins [45]. In order to avoid recurrences and repeat surgeries gynecologists are advised to prescribe a hormonal treatment postoperatively, which should be continued until the planned pregnancy, an approach that has been broadly adopted internationally [46-50].

However, 15.6-26.1% and 10-43.5% of the patients discontinue their hormonal therapy due to ineffectiveness or side effects, respectively [51]. This results in dissatisfaction and demotivation in many patients, possibly leading to repeat surgeries and thus contributing to the high health risks and costs related to endometriosis. In a recent study, we have identified risk factors for treatment non-response and discussed strategies to overcome this problem [52]. More specifically, genital bleeding during dienogest (DNG) treatment is a potential modifiable factor to improve response to DNG. A 5-7 days break of therapy, a course of nonsteroidal anti-inflammatory drugs or a short-term application of 1mg estradiol have been described as valid approaches [53-55]. Another way to interrupt the bleeding episode may be doubling the dose from 2 mg to 4 mg daily for 5-7 days. In our personal experience, we have found this regiment to be able to break through the uterine bleeding episode in certain patients. In contrast, in a multicenter study with 68 patients each treated with dienogest 1 mg, 2 mg and 4 mg during 24 weeks, the 4 mg dosage of dienogest (68.6%) was associated with more frequent vaginal bleeding episodes than the 2 mg dosage (55.2%). In both groups, the bleeding tended to improve over time [56]. Another option could be to treat patients with recurrent genital bleeding with a non-progestin based treatment, such as GnRHa in order to achieve amenorrhea and then switch to DNG [57]. Future studies are needed to assess which of the above-outlied strategies is the best possible approach. In any case, given the high possibility of irregular bleeding patterns especially at the beginning of the DNG therapy, it is recommended to prepare and reassure patients of possible bleeding as well as inform them about the abovementioned management approaches [54].

In the same study [52], we reported a high percentage of comorbidities in patients with endometriosis such as chronic pain out of the pelvis, and chronic gastrointestinal symptoms. For these patients a viscero-visceral-hyperalgesia has been described meaning that several co-existing visceral pain conditions in the same patient increase each other's symptoms [58]. Effective treatment of one condition

significantly improves symptoms from the other. Specifically, irritable bowel syndrome dietary treatment has been shown to improve dysmenorrhea [58]. Therefore, a holistic pain management with treatment of the viscero-visceral-hyperalgesia has the potential to increase the response to hormonal treatment and reduce the need for recurrent surgeries in endometriosis. A multidisciplinary approach should be considered in these patients including a pain specialist, nutritionist, and physiotherapist.

The frequent coexistence of endometriosis and adenomyosis is well known. The presence of adenomyosis in patients undergoing surgery for deep endometriosis has been reported to be as high as 48.7% [41]. Adenomyosis was also present in 79% of patients with history of infertility and endometriosis diagnosed by MRI [59]. Adenomyosis is not only correlated with pain persistence after endometriosis surgery [60] but also DNG non-response in patients with endometriosis [52]. In addition, it is well known that both endometriosis and adenomyosis correlate with a negative fertility outcome [61, 62]. Our studies showed that they are also negatively associated with adverse pregnancy and neonatal outcomes [63-65].

More specifically, endometriosis is associated with a higher risk of preterm birth, miscarriage, placenta previa, small for gestational age infants, and cesarean delivery [28]. Similar risks were described in pregnant women with surgically untreated rectovaginal endometriosis while an increased rate of complications during delivery was observed [33]. Interestingly, we showed that even after a previous excisional surgery for rectovaginal endometriosis the pregnancy risks remain unchanged. Importantly however, the surgery does not predispose to failed vaginal delivery. Except for a significantly higher postpartal blood loss in patients with endometriosis, all other delivery and neonatal risks were similar to the control patients [64]. Therefore, in patients with previously surgically treated rectovaginal endometriosis a vaginal delivery could be offered.

Our most recent systematic meta-analysis revealed that adenomyosis is associated with a higher risk of preterm delivery, preeclampsia, cesarean section, fetal malpresentation, low birth weight, and PPH [65]. However, as stated above, adenomyosis is very often coexistent with endometriosis. It is therefore unclear to which of the two conditions the above mentioned pregnancy risks should be attributed. To examine adenomyosis as an endometriosis-independent risk factor for adverse pregnancy outcomes we performed a sensitivity analysis including only studies matched for the existence of endometriosis. Consequently, we identified that adenomyosis is an endometriosis-independent risk factor for small for gestational age fetuses.

Our same study showed a negative association between adenomyosis and fertility outcome especially after short protocol downregulation in ART. This association is less significant or absent in ART with mixed or ultra-long GnRHa protocols but the results are not sufficient for a conclusive evaluation of the most proper ART protocol. Ongoing randomized controlled studies intend to provide reliable conclusive data on the possible protective role of ultra-long GnRHa downregulation in adenomyosis [38, 39].

However, adenomyosis is a very heterogenic disorder with varying extent of lesions, ranging from multiple lesions with diffuse myometrial hypertrophy to more

discrete focal lesions [66]. It is plausible that the impact of adenomyosis on the reproductive course is not always the same rather than dependent on the degree of the uterus involvement. Concerning the clinical pregnancy rate after ART two studies compared the effects of focal versus diffuse adenomyosis on clinical pregnancy rate [36, 67]. The pooled results gave a statistically non-significant OR of 1.36 favoring focal adenomyosis (CI: 0.67-2.75) [68]. Unfortunately, our objective to address this issue in relation to pregnancy and neonatal risks could not be adequately met due to the different diagnostic criteria and inadequate characterization of adenomyosis in the single studies. We strongly suggest that both for research and clinical purposes certain ultrasound or MRI criteria are systematically evaluated and used to adequately describe the extent of adenomyosis [69, 70].

In conclusion, the potentially protective role of the ultra-long downregulation protocols in relation to fertility outcome after ART in patients with adenomyosis needs further evaluation in randomized controlled studies. When a pregnancy is achieved, though, gynecologists should be aware of the associated pregnancy risks to indicate proper controls enabling an early diagnosis and treatment of possible complications.

Future research directions

The identification of reliable diagnostic, prognostic and predictive markers for endometriosis should be set as one of the priorities in the research society in order to achieve personalization in healthcare. This is a major demand on the medical community especially in endometriosis where several medical treatments have been repeatedly reported as ineffective or with significant side effects in a high number of patients. An evidence-based approach to personalise treatment would significantly improve clinical options and patient outcomes.

Better diagnostic tools

According to current guidelines, endometriosis-related pain can be empirically treated with adequate analgesia and combined oral contraceptives or progestins even prior to a laparoscopic diagnosis of endometriosis [16]. This approach is recommended due to the invasive nature of the laparoscopy, which is currently the gold-standard diagnostic tool. However, pain is not a very specific symptom for endometriosis and endometriosis-related pain varies significantly. As a result, some patients will receive a treatment for endometriosis although their pain is not endometriosis-related rather than related to another disorder. On the other hands, some patients with endometriosis will receive no treatment either because their pain is mild or because it may not seem indicative for endometriosis. Novel non-invasive diagnostic tools alternative to laparoscopy are needed so that endometriosis is diagnosed without the current delay.

Better prognostic tools

Besides the hormonal treatment of endometriosis, the laparoscopic excision of endometriotic lesions represents an important tool for the management of the disease. This is associated with a decreased overall pain, both at 6 and 12 months after surgery as well as fertility improvement in some cases [71, 72]. However, even after a complete removal of endometriotic lesions a high proportion of patients will

require additional surgery due to endometriosis recurrence with total recurrence rates of 21.5% and 40-50% at 2 and 5 years, respectively [44, 45, 73-75]. With our current work, the recurrence potential of different lesion subtypes has been studied while certain risk factors for recurrence have been identified [44, 45]. Further research is needed to identify prognostic markers to objectively evaluate the probability of recurrence. The presence or absence of such prognostic markers could be useful for the selection of patients for adjuvant hormonal treatment.

Better predictive tools

Reliable predictive markers that will evaluate the likelihood of response and tolerability to the available drugs are urgently needed since current hormonal treatments are accompanied by significant, unwanted side effects and in about 30% of the patients a non-adequate reduction in endometriosis-associated pain [51, 52]. Unfortunately, a reliable identification of which patients will respond, or tolerate the treatment is currently impossible even if we have recently identified certain risk factors [52].

The reason for the non-response to treatment in some patients is unclear, however variants in the lesion themselves may play a role. Somatic mutations, including cancer driver mutations, are present in endometriotic lesions. A landmark paper [76] reported somatic mutations in DIE in 79% of the 24 patients examined. In lesions from five patients (21%) known somatic cancer driver mutations in ARID1A, PIK3CA, KRAS, and PPP2R1A were identified even though the tissue remained benign. In a concurrent study of 24 OMA lesions, 4192 somatic mutations were found, including mutations in KRAS, PIK3CA, FBXW7, PPP2R1A and PIK3R1 that occurred in at least 3 or more samples and analysis of allele frequency indicated these mutations provided a growth advantage driving clonal expansion [77]. These studies raise the possibility that up to 20% of endometriosis lesions acquire somatic mutations that provide them the ability to grow in a suppressed estrogen environment. The increased activity of these lesions could lead to more intense symptoms.

Lesion transcriptomic variation is also a potential influence on treatment response. Our previous work established that inflammatory cytokines mediate endometriosis-associated pelvic pain [22, 78], that GnRH analogues and dienogest suppress peritoneal inflammation [23, 79] and also endometrial cellular proliferation [24]. Moreover, we found that the interaction between inflammation and secondary progestin targets could influence progestin activity [80]. Through the culmination of this work, we proposed that progesterone resistance in endometriotic lesions was not inherent to endometriotic lesions, but rather dependent on the composition of the inflammatory microenvironment. The progesterone receptor could be downregulated due to lesion exposure to the inflammation [81]. Interestingly, a recent study showed a direct correlation between progesterone receptors in endometriotic lesions and progestin response [82]. Endometriosis lesions however are a mixture of epithelial and stromal cells as well as infiltrating immune cells [83] and to fully understand potential influence of the transcriptome on treatment response it needs to be studied in these different cell types individually.

Ultimately, therefore, a resistance to progestin treatment may be driven by variations in local genomic, transcriptomic, or cell composition within the lesion. These variations could be predictive for the drug effectiveness. To better target

available treatments to endometriosis patients, it would be challenging to determine the relationship between local lesion variations and clinical responses.

Medical therapies with improved clinical profile

Current medical therapies are comprised of 16 marketed drugs from 7 different therapeutic classes. All therapeutics classes share the common mechanism of action of inducing a hypoestrogenic state. Novel drug therapies for endometriosis with improved tolerability and effectiveness, if possible, compatible with the desire to get pregnant are urgently needed.

We have observed an anti-inflammatory effect of both GnRHa [79] and dienogest [23], which could partially explain their effectiveness in endometriosis. It is possible that this effect is exerted via as yet unclear non-hormonal functions at local site rather than through their typical estrogen-suppressive function [84]. A better understanding of these mechanisms is needed. Optimising the anti-inflammatory components would create the potential to reduce oestrogen suppression and limit the adverse effects associated with it, while enhancing the anti-proliferative effect on the lesions themselves. One suggestion could be to screen novel compounds for inflammation modulating effects in an in-vitro environment using patient derived endometriotic epithelial and/or stromal cells. This could facilitate the development of novel therapeutics with improved clinical profile that would emphasize non-hormonal functions of GnRHa and progestins.

Role of different endometriosis or adenomyosis subtypes on fertility, pregnancy and delivery

Adenomyosis is a very heterogenic disorder with varying extent of lesions, ranging from multiple lesions with diffuse myometrial hypertrophy to more discrete focal lesions. It is possible therefore that the expected effects on the reproductive course differ significantly. Unfortunately, our objective to address this issue in our systematic meta-analysis [65] could not be adequately met due to the different diagnostic criteria and the inadequate characterization of adenomyosis in the single studies. Certain diagnostic criteria for adenomyosis have been proposed [85] and we strongly suggest that these are systematically used in future studies in order to investigate which adenomyosis characteristics, if any, are the most important for the reproductive course. These studies should ideally extend from fertility desire to postpartum period to investigate the role of specific adenomyosis subtypes and their treatment in every aspect of the reproductive course.

Approaches to counteract the negative impact of adenomyosis on the reproductive course

The possible positive effect of the ultra-long downregulation protocols on the fertility outcome prior to ART is very promising and deserves further evaluation. Ongoing studies are expected and if this is confirmed, a significant change in the current stimulation strategies is expected. Given recent advances in vitrification technology resulting in enhanced embryo survival and pregnancy rates [27], it could be plausible to firstly vitrify embryos at the blastocyst stage to confirm adequate development and then administer prolonged GnRHa before endometrial preparation with the aim to inactivate adenomyosis, reduce the uterine volume and maybe normalize some of the distorted endometrial functions. This would eliminate concerns regarding excessive ovarian suppression without affecting any possible beneficial impact on fertility outcomes provided by GnRHa. However, one has to

keep in mind that frozen blastocyst transfers are associated with a higher risk of preeclampsia (RR 3.13, 95% CI 1.06–9.30, p=0.029) [40]. This risk has to be weighed against the potential benefits of the above-described approach.

Moreover, it would be very interesting to examine the role of alternative treatments such as progestins prior to ART on the fertility outcome since they share a better tolerability profile and less ovarian suppression effect than GnRHa.

If the positive effects of the above medicines are confirmed it would be tempting to hypothesize that the adenomyosis-associated pregnancy and neonatal risks such as preeclampsia, placenta previa and intrauterine growth restriction would be also counteracted though a down-regulative medication prior to the conception. We suggest therefore, that all pregnancies resulting after ultra-long downregulation ART protocols are further followed during pregnancy and labor within well-designed controlled studies.

Recently, first trimester screening for late pregnancy complications has been widely adopted for all women since early intervention has the potential to reduce certain risks significantly [86]. As an example, treatment with low-dose aspirin in women at high risk for preterm preeclampsia according to the screening test results in a 60% risk reduction [87]. For the specific population of patients with adenomyosis or endometriosis it is unknown if the current first-trimester screening can accurately identify patients at risk and if early intervention reduces these risks; a hypothesis that deserves further evaluation. It is plausible to suppose, though, that the pathophysiologic mechanisms involved in the endometriosis/adenomyosis-associated preeclampsia are similar to the known preeclampsia mechanisms (distorted placentation). Therefore, we currently suggest that pregnant patients with adenomyosis/ endometriosis are screened and treated similarly to all other pregnant women without adenomyosis/endometriosis.

References:

1. Koninckx PR, Meuleman C, Demeyere S, Lesaffre E, Cornillie FJ. Suggestive evidence that pelvic endometriosis is a progressive disease, whereas deeply infiltrating endometriosis is associated with pelvic pain. *Fertility and sterility* 1991, 55: 759-65.

2. Brosens I, Gordts S, Benagiano G. Endometriosis in adolescents is a hidden, progressive and severe disease that deserves attention, not just compassion. *Human reproduction* 2013, 28: 2026-31.

3. Unger CA, Laufer MR. Progression of endometriosis in non-medically managed adolescents: a case series. *J Pediatr Adolesc Gynecol* 2011, 24: e21-3.

4. Hans Evers JL. Is adolescent endometriosis a progressive disease that needs to be diagnosed and treated? *Human reproduction* 2013, 28: 2023.

5. Harrison RF, Barry-Kinsella C. Efficacy of medroxyprogesterone treatment in infertile women with endometriosis: a prospective, randomized, placebo-controlled study. *Fertility and sterility* 2000, 74: 24-30.

6. Koh CE, Juszczyk K, Cooper MJ, Solomon MJ. Management of deeply infiltrating endometriosis involving the rectum. *Diseases of the colon and rectum* 2012, 55: 925-31.

7. Mabrouk M, Spagnolo E, Raimondo D, D'Errico A, Caprara G, Malvi D *et al.* Segmental bowel resection for colorectal endometriosis: is there a correlation between histological pattern and clinical outcomes? *Human reproduction* 2012, 27: 1314-9.

8. Anaf V, El Nakadi I, De Moor V, Coppens E, Zalcman M, Noel JC. Anatomic significance of a positive barium enema in deep infiltrating endometriosis of the large bowel. *World journal of surgery* 2009, 33: 822-7.

9. Badescu A, Roman H, Aziz M, Puscasiu L, Molnar C, Huet E *et al.* Mapping of bowel occult microscopic endometriosis implants surrounding deep endometriosis nodules infiltrating the bowel. *Fertility and sterility* 2016, 105: 430-4.e26.

10. Roman H, Hennetier C, Darwish B, Badescu A, Csanyi M, Aziz M *et al.* Bowel occult microscopic endometriosis in resection margins in deep colorectal endometriosis specimens has no impact on short-term postoperative outcomes. *Fertility and sterility* 2016, 105: 423-9.e7.

11. Roman H, Bridoux V, Tuech JJ, Marpeau L, da Costa C, Savoye G *et al.* Bowel dysfunction before and after surgery for endometriosis. *American journal of obstetrics and gynecology* 2013, 209: 524-30.

12. Fedele L, Bianchi S, Zanconato G, Bettoni G, Gotsch F. Long-term follow-up after conservative surgery for rectovaginal endometriosis. *American journal of obstetrics and gynecology* 2004, 190: 1020-4.

13. Nezhat C, Hajhosseini B, King LP. Laparoscopic management of bowel endometriosis: predictors of severe disease and recurrence. *JSLS : Journal of the Society of Laparoendoscopic Surgeons* 2011, 15: 431-8.

14. Guo S-W. Recurrence of endometriosis and its control. *Human reproduction update* 2009, 15: 441-61.

15. Vignali M, Bianchi S, Candiani M, Spadaccini G, Oggioni G, Busacca M. Surgical treatment of deep endometriosis and risk of recurrence. *Journal of minimally invasive gynecology* 2005, 12: 508-13.

16. Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nature Reviews Endocrinology* 2019, 15: 666-82.

17. Vercellini P, Somigliana E, Daguati R, Vigano P, Meroni F, Crosignani PG. Postoperative oral contraceptive exposure and risk of endometrioma recurrence. *American journal of obstetrics and gynecology* 2008, 198: 504.e1-5.

18. Lee SR, Yi KW, Song JY, Seo SK, Lee DY, Cho S *et al.* Efficacy and Safety of Long-Term Use of Dienogest in Women With Ovarian Endometrioma. *Reproductive sciences* 2018;25:341-6.

19. Somigliana E, Vercellini P, Vigano P, Benaglia L, Busnelli A, Fedele L. Postoperative medical therapy after surgical treatment of endometriosis: from adjuvant therapy to tertiary prevention. *Journal of minimally invasive gynecology* 2014, 21: 328-34.

20. Bergqvist A, Nejaty H, Fröysa B, Bruse C, Carlberg M, Sjöblom P *et al.* Production of interleukins 1beta, 6 and 8 and tumor necrosis factor alpha in separated and cultured endometrial and endometriotic stromal and epithelial cells. *Gynecologic and obstetric investigation* 2000, 50: 1-6.

21. Jung H, Toth PT, White FA, Miller RJ. Monocyte chemoattractant protein-1 functions as a neuromodulator in dorsal root ganglia neurons. *Journal of neurochemistry* 2008, 104: 254-63.

22. McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and nerve fiber interaction in endometriotic pain. *Trends in endocrinology and metabolism: TEM* 2015, 26: 1-10.

23. Nirgianakis K, Grandi G, McKinnon B, Bersinger N, Cagnacci A, Mueller M. Dienogest mediates midkine suppression in endometriosis. *Human reproduction* 2016, 31: 1981-6.

24. Grandi G, Mueller M, Bersinger N, Papadia A, Nirgianakis K, Cagnacci A *et al.* Progestin suppressed inflammation and cell viability of tumor necrosis factor-alpha-stimulated endometriotic stromal cells. *American journal of reproductive immunology* 2016, 76: 292-8.

25. Sallam HN, Garcia-Velasco JA, Dias S, Arici A. Long-term pituitary down-regulation before in vitro fertilization (IVF) for women with endometriosis. *The Cochrane database of systematic reviews* 2006: Cd004635.

26. Kaponis A, Chatzopoulos G, Paschopoulos M, Georgiou I, Paraskevaidis V, Zikopoulos K *et al.* Ultralong administration of gonadotropin-releasing hormone agonists before in vitro fertilization improves fertilization rate but not clinical pregnancy rate in women with mild endometriosis: a prospective, randomized, controlled trial. *Fertility and sterility* 2020, 113: 828-35.

27. Surrey ES. To suppress or not to suppress? If that is the question, has it been answered? *Fertility and sterility* 2020, 113: 763-4.

28. Zullo F, Spagnolo E, Saccone G, Acunzo M, Xodo S, Ceccaroni M *et al.* Endometriosis and obstetrics complications: a systematic review and meta-analysis. *Fertility and sterility* 2017, 108: 667-72.e5.

29. Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C *et al.* Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. *Human reproduction* 2010, 25: 265-74.

30. Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjaerven R, Vatten LJ. Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother. *Human reproduction* 2006, 21: 2353-8.

31. Shevell T, Malone FD, Vidaver J, Porter TF, Luthy DA, Comstock CH *et al.* Assisted reproductive technology and pregnancy outcome. *Obstet Gynecol* 2005, 106: 1039-45.

32. Committee Opinion No 671: Perinatal Risks Associated With Assisted Reproductive Technology. *Obstet Gynecol* 2016, 128: e61-8.

33. Exacoustos C, Lauriola I, Lazzeri L, De Felice G, Zupi E. Complications during pregnancy and delivery in women with untreated rectovaginal deep infiltrating endometriosis. *Fertility and sterility* 2016, 106: 1129-35.e1.

34. Thalluri V, Tremellen KP. Ultrasound diagnosed adenomyosis has a negative impact on successful implantation following GnRH antagonist IVF treatment. *Human reproduction* 2012, 27: 3487-92.

35. Imaoka I, Ascher SM, Sugimura K, Takahashi K, Li H, Cuomo F *et al.* MR imaging of diffuse adenomyosis changes after GnRH analog therapy. *Journal of magnetic resonance imaging : JMRI* 2002, 15: 285-90.

36. Park CW, Choi MH, Yang KM, Song IO. Pregnancy rate in women with adenomyosis undergoing fresh or frozen embryo transfer cycles following gonadotropin-releasing hormone agonist treatment. *Clinical and experimental reproductive medicine* 2016, 43: 169-73.

37. Niu Z, Chen Q, Sun Y, Feng Y. Long-term pituitary downregulation before frozen embryo transfer could improve pregnancy outcomes in women with adenomyosis. *Gynecological Endocrinology* 2013, 29: 1026-30.

38. Modified Downregulation for Women With Adenomyosis of the Uterus Prior to Frozen-thawed Embryo Transfer.

39. Impact of Ultra-long Versus Long Down-regulation Protocol on IVF/ICSI in *Adenomyosis.*

40. Wei D, Liu JY, Sun Y, Shi Y, Zhang B, Liu JQ *et al.* Frozen versus fresh single blastocyst transfer in ovulatory women: a multicentre, randomised controlled trial. *Lancet (London, England)* 2019, 393: 1310-8.

41. Lazzeri L, Di Giovanni A, Exacoustos C, Tosti C, Pinzauti S, Malzoni M *et al.* Preoperative and Postoperative Clinical and Transvaginal Ultrasound Findings of Adenomyosis in Patients With Deep Infiltrating Endometriosis. *Reproductive sciences* 2014, 21: 1027-33.

42. Naftalin J, Hoo W, Pateman K, Mavrelos D, Holland T, Jurkovic D. How common is adenomyosis? A prospective study of prevalence using transvaginal ultrasound in a gynaecology clinic. *Human reproduction* 2012, 27: 3432-9.

43. Sharma S, Bathwal S, Agarwal N, Chattopadhyay R, Saha I, Chakravarty B. Does presence of adenomyosis affect reproductive outcome in IVF cycles? A retrospective analysis of 973 patients. *Reproductive biomedicine online* 2019, 38: 13-21.

44. Nirgianakis K, Ma L, McKinnon B, Mueller MD. Recurrence Patterns after Surgery in Patients with Different Endometriosis Subtypes: A Long-Term Hospital-Based Cohort Study. *Journal of Clinical Medicine* 2020, 9: 496.

45. Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller MD. Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence. *Acta obstetricia et gynecologica Scandinavica* 2014, 93: 1262-7.

46. Vercellini P, S DEM, Somigliana E, Buggio L, Frattaruolo MP, Fedele L. Long-term adjuvant therapy for the prevention of postoperative endometrioma recurrence: a systematic review and meta-analysis. Acta obstetricia et gynecologica Scandinavica 2013, 92: 8-16.

47. Koga K, Takamura M, Fujii T, Osuga Y. Prevention of the recurrence of symptom and lesions after conservative surgery for endometriosis. *Fertility and sterility* 2015, 104: 793-801.

48. Seracchioli R, Mabrouk M, Manuzzi L, Vicenzi C, Frascà C, Elmakky A *et al.* Post-operative use of oral contraceptive pills for prevention of anatomical relapse or symptom-recurrence after conservative surgery for endometriosis. *Human reproduction* 2009, 24: 2729-35.

49. Leyland N, Casper R, Laberge P, Singh SS. Endometriosis: diagnosis and management. *Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC* 2010, 32: S1-32.

50. Kuznetsov L, Dworzynski K, Davies M, Overton C. Diagnosis and management of endometriosis: summary of NICE guidance. *Bmj* 2017, 358: j3935.

51. Becker CM, Gattrell WT, Gude K, Singh SS. Reevaluating response and failure of medical treatment of endometriosis: a systematic review. *Fertility and sterility* 2017, 108: 125-36.

52. Nirgianakis K, Vaineau C, Agliati L, McKinnon B, Gasparri ML, Mueller MD. Risk factors for non-response and discontinuation of Dienogest in endometriosis patients: A cohort study. *Acta obstetricia et gynecologica Scandinavica* 2020.
53. Romer T. Long-term treatment of endometriosis with dienogest: retrospective analysis of efficacy and safety in clinical practice. *Archives of gynecology and obstetrics* 2018, 298: 747-53.

54. Murji A, Biberoğlu K, Leng J, Mueller MD, Römer T, Vignali M *et al.* Use of dienogest in endometriosis: a narrative literature review and expert commentary. *Current medical research and opinion* 2020, 36: 895-907.

55. Tantiwattanakul P, Taneepanichskul S. Effect of mefenamic acid on controlling irregular uterine bleeding in DMPA users. *Contraception* 2004, 70: 277-9.

56. Kohler G, Faustmann TA, Gerlinger C, Seitz C, Mueck AO. A dose-ranging study to determine the efficacy and safety of 1, 2, and 4mg of dienogest daily for endometriosis. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics* 2010, 108: 21-5.

57. Kitawaki J, Kusuki I, Yamanaka K, Suganuma I. Maintenance therapy with dienogest following gonadotropin-releasing hormone agonist treatment for endometriosis-associated pelvic pain. *European journal of obstetrics, gynecology, and reproductive biology* 2011, 157: 212-6.

58. Giamberardino MA, Costantini R, Affaitati G, Fabrizio A, Lapenna D, Tafuri E *et al.* Viscero-visceral hyperalgesia: characterization in different clinical models. *Pain* 2010, 151: 307-22.

59. Kunz G, Beil D, Huppert P, Noe M, Kissler S, Leyendecker G. Adenomyosis in endometriosis--prevalence and impact on fertility. Evidence from magnetic resonance imaging. *Human reproduction* 2005, 20: 2309-16.

60. Parker JD, Leondires M, Sinaii N, Premkumar A, Nieman LK, Stratton P. Persistence of dysmenorrhea and nonmenstrual pain after optimal endometriosis surgery may indicate adenomyosis. *Fertility and sterility* 2006, 86: 711-5.

61. de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. *Lancet (London, England)* 2010, 376: 730-8.

62. Chapron C, Vannuccini S, Santulli P, Abrao MS, Carmona F, Fraser IS *et al.* Diagnosing adenomyosis: an integrated clinical and imaging approach. *Human reproduction update* 2020.

63. Gasparri ML, Nirgianakis K, Taghavi K, Papadia A, Mueller MD. Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis. *Archives of gynecology and obstetrics* 2018, 298: 27-34.

64. Nirgianakis K, Gasparri ML, Radan AP, Villiger A, McKinnon B, Mosimann B *et al.* Obstetric complications after laparoscopic excision of posterior deep infiltrating endometriosis: a case-control study. *Fertility and sterility* 2018, 110: 459-66.

65. Nirgianakis K, Kalaitzopoulos DR, Schwartz ASK, Spaanderman M, Kramer BW, Mueller MD *et al.* Fertility, pregnancy and neonatal outcomes of patients with adenomyosis: a systematic review and meta-analysis. *Reproductive biomedicine online* 2020.

66. Chapron C, Tosti C, Marcellin L, Bourdon M, Lafay-Pillet MC, Millischer AE *et al.* Relationship between the magnetic resonance imaging appearance of adenomyosis and endometriosis phenotypes. *Human reproduction* 2017, 32: 1393-401.

67. Benaglia L, Cardellicchio L, Leonardi M, Faulisi S, Vercellini P, Paffoni A *et al.* Asymptomatic adenomyosis and embryo implantation in IVF cycles. Reproductive biomedicine online 2014;29:606-11.

68. Younes G, Tulandi T. Effects of adenomyosis on in vitro fertilization treatment outcomes: a meta-analysis. *Fertility and sterility* 2017, 108: 483-90 e3.

69. Van den Bosch T, de Bruijn AM, de Leeuw RA, Dueholm M, Exacoustos C, Valentin L *et al.* Sonographic classification and reporting system for diagnosing adenomyosis. Ultrasound in obstetrics & gynecology : *the official journal of the International Society of Ultrasound in Obstetrics and Gynecology* 2019, 53: 576-82.

70. Bazot M, Darai E. Role of transvaginal sonography and magnetic resonance imaging in the diagnosis of uterine adenomyosis. *Fertility and sterility* 2018, 109: 389-97.

71. Marcoux S, Maheux R, Bérubé S. Laparoscopic surgery in infertile women with minimal or mild endometriosis. Canadian Collaborative Group on Endometriosis. *The New England journal of medicine* 1997;337:217-22.

72. Duffy JM, Arambage K, Correa FJ, Olive D, Farquhar C, Garry R *et al.* Laparoscopic surgery for endometriosis. *The Cochrane database of systematic reviews* 2014: Cd011031.

73. Cea Soriano L, Lopez-Garcia E, Schulze-Rath R, Garcia Rodriguez LA. Incidence, treatment and recurrence of endometriosis in a UK-based population analysis using data from The Health Improvement Network and the Hospital Episode Statistics database. *The European journal of contraception & reproductive health care : the official journal of the European Society of Contraception* 2017, 22: 334-43.

74. Guo SW. Recurrence of endometriosis and its control. *Human reproduction update* 2009, 15: 441-61.

75. Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Vigano P, Fedele L. The effect of surgery for symptomatic endometriosis: the other side of the story. *Human reproduction update* 2009 ,15: 177-88.

76. Anglesio MS, Papadopoulos N, Ayhan A, Nazeran TM, Noë M, Horlings HM *et al.* Cancer-Associated Mutations in Endometriosis without Cancer. *The New England journal of medicine* 2017, 376: 1835-48.

77. Suda K, Nakaoka H, Yoshihara K, Ishiguro T, Tamura R, Mori Y *et al.* Clonal Expansion and Diversification of Cancer-Associated Mutations in Endometriosis and Normal Endometrium. *Cell reports* 2018, 24: 1777-89.

78. McKinnon B, Bersinger NA, Wotzkow C, Mueller MD. Endometriosis-associated nerve fibers, peritoneal fluid cytokine concentrations, and pain in endometriotic lesions from different locations. *Fertility and sterility* 2012, 97: 373-80.

79. Nirgianakis K, Bersinger NA, McKinnon B, Kostov P, Imboden S, Mueller MD. Regression of the inflammatory microenvironment of the peritoneal cavity in women with endometriosis by GnRHa treatment. *European journal of obstetrics, gynecology, and reproductive biology* 2013, 170: 550-4.

80. Grandi G, Mueller MD, Papadia A, Kocbek V, Bersinger NA, Petraglia F *et al.* Inflammation influences steroid hormone receptors targeted by progestins in endometrial stromal cells from women with endometriosis. *Journal of reproductive immunology* 2016, 117:30-8.

81. McKinnon B, Mueller M, Montgomery G. Progesterone Resistance in Endometriosis: an Acquired Property? *Trends in endocrinology and metabolism: TEM* 2018, 29: 535-48.

82. Flores VA, Vanhie A, Dang T, Taylor HS. Progesterone Receptor Status Predicts Response to Progestin Therapy in Endometriosis. *The Journal of clinical endocrinology and metabolism* 2018, 103: 4561-8.

83. Riccio L, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. *Best practice & research Clinical obstetrics & gynaecology* 2018, 50: 39-49.

84. Grandi G, Mueller M, Bersinger NA, Cagnacci A, Volpe A, McKinnon B. Does dienogest influence the inflammatory response of endometriotic cells? A systematic review. *Inflammation Research* 2016, 65: 183-92.

85. Gordts S, Grimbizis G, Campo R. Symptoms and classification of uterine adenomyosis, including the place of hysteroscopy in diagnosis. *Fertility and sterility* 2018, 109: 380-8 e1.

86. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H *et al.* The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: A pragmatic guide for first-trimester screening and prevention. International journal of gynaecology and obstetrics: the official organ of the *International Federation of Gynaecology and Obstetrics* 2019, 145 Suppl 1: 1-33.

87. Rolnik DL, Wright D, Poon LC, O'Gorman N, Syngelaki A, de Paco Matallana C *et al.* Aspirin versus Placebo in Pregnancies at High Risk for Preterm Preeclampsia. *The New England journal of medicine* 2017, 377: 613-22.

Summary

The impact of endometriosis and adenomyosis on the female reproductive system: risks and management approaches

Endometriosis and adenomyosis affect a significant proportion of reproductive-aged women causing a serious disease burden. National action plans have been declared with the aim to reduce the impact and burden of disease at individual and population levels. The chronic nature and heterogeneity of the disease, the frequent recurrences after surgery as well as the limitations of current medical treatments represent significant challenges. The objective of this research, therefore, was (1) to investigate the recurrence potential of different endometriosis lesion subtypes and identify risk factors of recurrence, (2) to evaluate the effect of current medical treatments on the endometriosis-mediated inflammation and (3) to examine the effects of endometriosis and adenomyosis on female fertility and pregnancy.

To address the first objective (1), we examined the evolution of different endometriosis lesion subtypes and compared the time to recurrence in a large cohort of patients who underwent surgery for endometriosis in the Department of Gynecology and Obstetrics, University of Bern. Similarly, we evaluated several clinical and histological parameters as possible risk factors for disease recurrence in a cohort of patients who underwent laparoscopic segmental bowel resection due to bowel endometriosis. We detected no difference in the recurrence potential between superficial peritoneal (SUP), ovarian (OMA) and deep infiltrating endometriosis (DIE). However, a high percentage of patients presented with more severe lesions subtypes, particularly DIE at recurrence.

To address the second objective (2), we measured the concentration of several biomarkers in the peritoneal fluid of patients undergoing surgery for endometriosis. We then compared them between patients with and without GnRHa treatment at the time of the surgery. Concerning the effect of GnRHa on the local microenvironment of the peritoneal cavity, we observed a significant regression of four biomarkers (IL-8, PAPP-A, glycodelin-A and midkine) in patients who had been treated with GnRHa prior to endometriosis surgery.

For the third objective (3), we performed two systematic meta-analyses and a matched case-control study. Firstly, we examined the association of endometriosis with placental disorders. More specifically, we compared the incidence of placental disorders in women with and without endometriosis in a systematic meta-analysis. The risk of placenta previa after assisted reproductive technology (ART) was found threefold higher in patients with than without endometriosis and this difference was statistically significant. Moreover, we examined the association of endometriosis with pregnancy complications in the specific population of patients with previous complete endometriosis excision. A 1:3 case-control study was performed while the case and control groups were matched for age, parity, previous cesarean section, and mode of conception. Patients with previously excised posterior DIE, similarly to women with endometriosis in general, were found to have a statistically significant increased risk of placenta previa, gestational hypertension, and IUGR compared with women without endometriosis. Moreover, the possibility of successful vaginal birth, if attempted, was high and similar to women without endometriosis. Except for a higher postpartum blood loss in the endometriosis group, all other delivery and neonatal risks were similar. Finally, we conducted a systematic meta-analysis including all observational studies comparing the

reproductive course of patients with and without adenomyosis. Detailed sensitivity analyses were performed to address specific aims and certain study limitations. Adenomyosis is associated with a significantly lower clinical pregnancy rate and higher miscarriage rate after ART, especially when a short GnRH agonist or antagonist protocol is administered for ovarian stimulation. Moreover, adenomyosis is associated with a higher risk of preterm delivery, preeclampsia, caesarean section, fetal malpresentation, SGA, low birth weight, and PPH. The association could be confirmed after adjustment of these outcomes for age and mode of conception.

The trend towards more severe endometriosis subtypes at recurrence suggests disease progression, which may occur overtime. Risk factors for recurrence include positive resection margins after surgery for bowel endometriosis as well as young age. Therefore, adjuvant hormonal therapy to avoid recurrence and potential lesion progression should be suggested, especially in patients with the above risk factors. Given the limitations of current hormonal therapeutics, though, research on novel therapies is urgently needed. The observed anti-inflammatory effect of GnRHa might be exerted via as yet unclear non-hormonal functions at local site rather than through their typical estrogen-suppressive function. A better understanding of these mechanisms could result in the development of novel medical treatments with better effectiveness and tolerability. Finally, endometriosis and adenomyosis are associated not only with worse fertility outcomes but also with a higher risk of pregnancy complications. These are mostly the result of either distorted placental function (IUGR, preeclampsia) or position (placenta previa, PPH). Previous endometriosis surgery neither reduces nor creates additional risks. Ultra-long GnRHa stimulation protocols might improve the fertility outcome in patients with adenomyosis, an observation that needs further investigation in prospective trials.

Nederlandse samenvatting
Summary in Dutch

De impact van endometriose en adenomyose op het vrouwelijke reproductief systeem: risico's en managementbenaderingen

Endometriose en adenomyose treft een aanzienlijk deel van de vrouwen in de vruchtbare leeftijd en veroorzaakt een substantiële ziektelast. Er zijn nationale actieplannen opgesteld met als doel de impact en de ziektelast op individueel en bevolkingsniveau te verminderen. De chronische aard en heterogeniteit van de ziekte, de frequente recidieven na een operatie en de beperkingen van de huidige medische behandelingen vormen een grote uitdaging. Het doel van dit onderzoek was daarom (1) om het herhaalkans van verschillende endometriose-laesie subtypen te onderzoeken en risicofactoren voor herhaling te identificeren, (2) om het effect van huidige medische behandelingen op de endometriose-gemedieerde ontsteking te evalueren en (3) om de effecten van endometriose en adenomyose op de vruchtbaarheid en zwangerschap uitkomsten van vrouwen onderzoeken.

Om de eerste vraag te beantwoorden, onderzochten we de ontwikkeling van verschillende subtypen van endometriose-laesies en vergeleken we de tijd tot recidief in een groot cohort van patiënten die een operatie voor endometriose ondergingen in de afdeling Gynaecologie en Verloskunde, Universiteit van Bern. Daarnaast hebben we verschillende klinische en histologische parameters geëvalueerd als mogelijke risicofactoren voor het terugkeren van de ziekte in een cohort van patiënten die een laparoscopische segmentale darmresectie ondergingen vanwege endometriose van de darm. We ontdekten geen verschil in het herhalingspotentieel tussen oppervlakkige peritoneale (SUP), ovariële (OMA) en diep infiltrerende endometriose (DIE). Een hoog percentage patiënten vertoonde echter de meer ernstige laesie-subtypen, met name bij DIE bij recidivering.

Om de tweede vraag te onderzoeken, maten we de concentratie van verschillende inflammatoire biomarkers in het peritoneale vocht van patiënten die een operatie voor endometriose ondergingen. We vergeleken ze vervolgens tussen patiënten met en zonder gonadodrophin-releasing hormone agonists (GnRHa)-behandeling op het moment van de operatie. We zagen dat GnRHa behandeling voorafgaand aan de ingreep op de lokale micro-omgeving van de peritoneale holte een significante regressie van vier inflammatoire biomarkers (IL-8, PAPP-A, glycodeline-A en midkine) lieten zien.

Voor de derde vraag hebben we twee systematische meta-analyses en een matchende case-control studie uitgevoerd. Allereest onderzochten we de associatie van endometriose met placenta-aandoeningen. We vergeleken hiervoor de incidentie van placenta-aandoeningen bij vrouwen met en zonder endometriose in een systematische review en meta-analyse. Het risico op placenta previa na artificiële reproductieve technieken (ART) werd driemaal hoger gevonden bij patiënten met dan zonder endometriose en dit verschil was statistisch significant. Daarnaast onderzochten we de associatie van endometriose met zwangerschapscomplicaties in de specifieke populatie patiënten met eerdere volledige endometriose-verwijdering. Een 1:3 case-control studie werd uitgevoerd waarbij de casus- en controle-groep werden gematcht op leeftijd, pariteit, eerdere keizersnede en wijze van conceptie. Patiënten met eerder operatief verwijderde posterieure DIE bleken, vergelijkbaar met vrouwen met endometriose in het algemeen, een statistisch significant verhoogd risico op voorliggende placenta (placenta previa), zwangerschapshypertensie en relatief en absoluut gering geboorte

gewicht te hebben ten opzichte van vrouwen zonder endometriose. De kans op een succesvolle vaginale bevalling, indien nagestreefd, was hoog en vergelijkbaar met die van vrouwen zonder endometriose. Behalve meer bloedverlies na de bevalling in de endometriosegroep, waren alle andere bevallingsrisico's en neonatale risico's vergelijkbaar. Ten slotte hebben we een systematische review en meta-analyse uitgevoerd, inclusief alle observationele studies, waarbij het reproductieve beloop van patiënten met en zonder adenomyose werden vergeleken. We voegden gevoeligheidsanalyses toe om specifieke doelen en bepaalde studiebeperkingen te compenseren. Adenomyose is geassocieerd met een significant lager klinisch zwangerschapspercentage en hoger miskraampercentage na ART, vooral wanneer een kort GnRH-agonist- of -antagonistprotocol wordt toegediend voor ovariële stimulatie. Bovendien wordt adenomyose geassocieerd met een hoger risico op vroeggeboorte, pre-eclampsie, keizersnede, foetale malpresentatie, relatief gering geboorte gewicht (SGA), laag geboortegewicht en fluxus post-partum. Dit verband bleef ook aanwezig na mede weging van leeftijd en wijze van conceptie.

De trend naar meer ernstige endometriose-subtypes bij recidive suggereert ziekteprogressie gedurende de tijd. Risicofactoren voor recidief zijn onder meer positieve snijvlakken na operatie voor endometriose van de darm en jonge leeftijd. Daarom zou aanvullende hormoontherapie, vooral bij patiënten met de bovengenoemde risicofactoren, kunnen worden overwogen om herhaling en mogelijke progressie van de endometriose haarden te voorkomen. Gezien de beperkingen van de huidige hormonale therapieën is onderzoek naar nieuwe therapieën echter dringend noodzakelijk. Het waargenomen ontstekingsremmende effect van GnRHa zou kunnen worden uitgeoefend via een nog onduidelijke niet-hormonale lokaal effect in plaats van via hun typische oestrogeen-onderdrukkende functie. Een beter begrip van deze mechanismen zou kunnen resulteren in de ontwikkeling van nieuwe medische behandelingen met een betere effectiviteit en tolerantie.

Ten slotte predisponeren endometriose en adenomyose niet alleen tot verminderde vruchtbaarheid, maar ook een hoger risico op zwangerschapscomplicaties. Deze zijn meestal het gevolg van een verstoorde placentafunctie (SGA, pre-eclampsie), positie (placenta previa, toegenomen bloedverlies rondom de bevalling), vroeggeboorte en gering geboorte gewicht. Eerdere endometriosechirurgie vermindert noch creëert extra risico's. Ultra-lange GnRHa-stimulatieprotocollen zouden het vruchtbaarheidsresultaat bij patiënten met adenomyose kunnen verbeteren, een observatie die verder onderzocht moet worden in prospectieve onderzoeken.

Impact

Impact

Endometriosis is a very prevalent disorder in women of premenopausal age. Due to the associated chronic pain and infertility it often leads to a significant reduction in quality of life (1). Moreover, the economic impact is substantial, as chronic and debilitating pain from endometriosis may hinder work productivity, while infertility can cause major psychosocial, emotional and financial strain to affected women and their partners (2). As a result, national action plans have been declared with the aim to improve the quality of life for individuals living with endometriosis, including a reduction in the impact and burden of disease at individual and population levels (3).

Adenomyosis is another similar gynecological disease associated with abnormal uterine bleeding, pelvic pain and infertility. It is characterized by the presence of endometrial-like tissue in the myometrium. Like endometriosis, it can lead to a significant reduction in quality of life. Moreover, it is often coexistent with endometriosis, which makes the treatment approach even more challenging.

We believe that, concerning endometriosis and adenomyosis, our current work may have a significant impact on patients, physisians and research society, which could be devided in the three following categories.

Firstly, we confirmed the high risk of endometriosis recurrence after surgery and showed that this is independent of the endometriosis lesion subtype (superficial lesions, ovarian lesions, deep-infiltrating lesions). The median time to recurrence was 30 months after surgery. Moreover, a significant proportion of the patients presented more severe lesions at recurrence (deep iflitrating lesions), which suggests endometriosis progression over time might occur. The recurrence risk was higher in young patients and if residual endometriosis tissue was identified on the margins of resected bowel. These findings support long-term adjuvant hormonal treatments, which have the potential to reduce the reccurence risk (4-6). Therefore, we believe our work will further encourage physisicans and patients to accept such long-term treatments. The benefits of a broad implementation and acceptance of this approach will be less recurrent surgeries with all the advantages related to that (lower risk of complications, lower health care costs). Our work encourages researchers to further investigate the mechanisms related to endometriosis recurrence as well as effectiveness and tolerance to specific treatments. This would aim to identify prognostic and predictive tools to allow for a personalized disease management.

Secondly, endometriosis is an estrogen-dependent disorder with a significant inflammatory nature. The inflammatory nature is one of the reasons for pelvic pain and infertility. GnRHa is a group of drugs acting in endometriosis by inducing a hypo-estrogenic state, which resemples menopause. We showed that GnRHa also mediate a significant regression of the inflammatory microenvironment of endometriotic lesions. By significantly reducing inflammatory cytokines and growth factors in the peritoneal cavity, GnRHa may contribute to pain relief in more ways than just the induction of a hypo-estrogenic state. Directly targeting some of these factors with non-hormonal treatments may achieve the same anti-inflammatory effect while avoiding the significant side effects associated with hormonal treatment (hot flushes, sleep disorders, decreased libido, mood disorders).

The development of novel drugs for endometriosis with improved effectiveness and tolerability is urgent and the above findings may be a first step to this direction.

Finally, it has been recently shown that endometriosis and adenomyosis are associated with several pregnancy complications. We showed that the higher risk of placental disorders is independent of the mode of conception in endometriosis and that a previous excision of endometriosis neither reduces nor increases the risk of pregnancy complications. More importantly, women with previous surgery for deep-infiltrating endometriosis had a similar possibility of successful vaginal birth, if attempted, to women without endometriosis. Concerns that the surgery for deep-infiltrating endometriosis with or without bowel or vaginal involvement may predispose to failed vaginal delivery are refuted by our study. This is valuable information to both physicians and patients to decide on the delivery method. Another important finding of our studies is that adenomyosis is associated with a significantly lower clinical pregnancy rate and higher miscarriage rate after ART, especially when a short GnRH agonist or antagonist protocol is administered for ovarian stimulation. On the contrary, an ultra-long GnRHa protocol might be capable of ameliorating these risks. If this is confirmed in prospective studies, it will be a very important tool to treat the adenomyosis-associated infertility. Finally, adenomyosis is associated with a higher risk of preterm delivery, preeclampsia, caesarean section, fetal malpresentation, SGA, low birth weight, and PPH. The association could be confirmed after adjustment of these outcomes for age and mode of conception. Gynecologists should be aware of these risks to indicate proper controls enabling an early diagnosis and treatment of possible complications.

References:

1. Vercellini P, Fedele L, Aimi G, Pietropaolo G, Consonni D, Crosignani PG. Association between endometriosis stage, lesion type, patient characteristics and severity of pelvic pain symptoms: a multivariate analysis of over 1000 patients. Human reproduction 2007;22:266-71.

2. Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I et al. The burden of endometriosis: costs and quality of life of women with endometriosis and treated in referral centres. Human reproduction 2012;27:1292-9.

3. Australia Co. National Action Plan for Endometriosis. In: Health Do, ed., 2018.

4. Vercellini P, Somigliana E, Daguati R, Vigano P, Meroni F, Crosignani PG. Postoperative oral contraceptive exposure and risk of endometrioma recurrence. American journal of obstetrics and gynecology 2008;198:504.e1-5.

5. Lee SR, Yi KW, Song JY, Seo SK, Lee DY, Cho S et al. Efficacy and Safety of Long-Term Use of Dienogest in Women With Ovarian Endometrioma. Reproductive sciences 2018;25:341-6.

6. Somigliana E, Vercellini P, Vigano P, Benaglia L, Busnelli A, Fedele L. Postoperative medical therapy after surgical treatment of endometriosis: from adjuvant therapy to tertiary prevention. Journal of minimally invasive gynecology 2014;21:328-34.

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Publications

Publications

THIS THESIS

Nirgianakis K, Ma L, McKinnon B, Mueller MD. Recurrence Patterns After Surgery in Patients With Different Endometriosis Subtypes: A Long-Term Hospital-Based Cohort Study. J Clin Med. 9:496, 2020

Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller MD. Laparoscopic management of bowel endometriosis: resection margins as a predictor of recurrence. Acta Obstet Gynecol Scand 93:1262–7, 2014.

Nirgianakis K, Bersinger NA, McKinnon B, Kostov P, Imboden S, Mueller MD. Regression of the inflammatory microenvironment of the peritoneal cavity in women with endometriosis by GnRHa treatment. Eur. J. Obstet. Gynecol. Reprod. Biol. 170:550–4, 2013.

Gasparri ML., **Nirgianakis K**, Taghavi K, Papadia A, Mueller MD. Placenta previa and placental abruption after assisted reproductive technology in patients with endometriosis: a systematic review and meta-analysis. Arch. Gynecol. Obstet. 298:27–34, 2018.

Nirgianakis K, Gasparri ML, Radan AP, Villiger A, McKinnon B, Mosimann B, Papadia A, Mueller MD. Obstetric complications after laparoscopic excision of posterior deep infiltrating endometriosis: a case-control study. Fertil. Steril. 110:459–466, 2018.

Nirgianakis K, Kalaitzopoulos DR, Schwartz ASK, Spaanderman M, Kramer BW, Mueller MD, Mueller M. Fertility, pregnancy and neonatal outcomes of patients with adenomyosis: a systematic review and meta-analysis. Reprod Biomed Online. 2021; 42:185-206. doi: 10.1016/j.rbmo.2020.09.023.

NOT INCULDED IN THIS THESIS

ENDOMETRIOSIS- CLINICAL PRESENTATION AND RECURRENCE

Knabben L, Imboden S, Fellmann B, **Nirgianakis K**, Kuhn A, Mueller MD. Urinary tract endometriosis in patients with deep infiltrating endometriosis: prevalence, symptoms, management, and proposal for a new clinical classification. Fertil. Steril. 103:147–52, 2015.

Nirgianakis K, Lanz S, Imboden S, Worni M, Mueller MD. Coagulation-Induced Diaphragm Fenestrations after Laparoscopic Excision of Diaphragmatic Endometriosis. J Minim Invasive Gynecol 25:771–772, 2018

Siegenthaler F, Knabben L, Mohr S, **Nirgianakis K**, Imboden S, Mueller MD. Visualization of Endometriosis With Laparoscopy and Near-Infrared Optics With Indocyanine Green. Acta Obstet Gynecol Scand. 99:591-597, 2020.

Nirgianakis K, Vaineau C, Agliati L, McKinnon B, Gasparri ML, Mueller M. Risk factors for non-response and discontinuation of Dienogest in endometriosis patients: a cohort study. Acta Obstet Gynecol Scand 2020. doi: 10.1111/aogs.13969. Online ahead of print.

ENDOMETRIOSIS- PATHOPHYSIOLOGY AND THERAPEUTICS

Mckinnon B, Mueller MD, **Nirgianakis K**, Bersinger NA. Comparison of ovarian cancer markers in endometriosis favours HE4 over CA125. Mol Med Rep 12:5179–84: 2015.

Nirgianakis K, Grandi G, McKinnon B, Bersinger N, Cagnacci A, Mueller M.. Dienogest mediates midkine suppression in endometriosis. Hum. Reprod. 31:1981–6: 2016.

Grandi G, Mueller M, Bersinger N, Papadia A, **Nirgianakis K**, Cagnacci A, McKinnon B. Progestin suppressed inflammation and cell viability of tumor necrosis factor-α-stimulated endometriotic stromal cells. Am. J. Reprod. Immunol. 76:292–8, 2016.

McKinnon BD, Kocbek V, **Nirgianakis K**, Bersinger NA, Mueller MD. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. Hum. Reprod. Update 22:382–403, 2016.

Nirgianakis K, McKinnon B, Ma L, Imboden S, Bersinger N, Mueller M. Peritoneal fluid biomarker in patients with endometriosis: a cross sectional study. Hormonal Molecular Biology and Clinical Investigation. Online ahead of print.

Nirgianakis K, Egger K, Kalaitzopoulos DR, Lanz S, Bally L, Mueller MD. Effectiveness of dietary interventions in the treatment of endometriosis: a systematic review. Reprod Sciences. doi: 10.1007/s43032-020-00418-w. Online ahead of print.

Andrieu T, Chicca A, Pellegata D, Bersinger NA, Imboden S, **Nirgianakis K**, Gertsch J, Mueller MD. Association of endocannabinoids with pain in endometriosis. Pain. doi: 10.1097/j.pain.0000000000002333. Online ahead of print. 2021.

Imboden S, Bollinger Y, Härmä K, Knabben L, Fluri M, **Nirgianakis K**, Mohr S, Kuhn A, Mueller MD. Predictive Factors for Voiding Dysfunction after Surgery for Deep Infiltrating Endometriosis. J Minim Invasive Gynecol. 28:1544-1551. 2021

Kalaitzopoulos DR, Lempesis IG, Samartzis N, Kolovos G, Dedes I, Daniilidis A, **Nirgianakis K**, Leeners B, Goulis DG, Samartzis EP. Leptin concentrations in endometriosis: A systematic review and meta-analysis. J Reprod Immunol. PMID: 34126469, 2021.

Kanellos P, **Nirgianakis K**, Siegenthaler F, Vetter C, Mueller MD, Imboden S. Postoperative Pain Is Driven by Preoperative Pain, Not by Endometriosis. J Clin Med. 10: 4727, 2021.

OTHERS

Papadia A, Imboden S, Mohr S, Lanz S, **Nirgianakis K**, Mueller MD. Indocyanine Green Fluorescence Imaging in the Surgical Management of an Iatrogenic Lymphatic Fistula: Description of a Surgical Technique. J Minim Invasive Gynecol 22:1304–6: 2015.

Nirgianakis K, Bersinger NA, McKinnon B, Kostov P, Imboden S, Mueller MD. Interdisziplinäre Behandlung des Endometriumkarzinoms. Schweiz Med Forum 15:1050-1054, 2015.

Nirgianakis K, Oehler R, Mueller M. The Rendez-vous technique for treatment of caesarean scar defects: a novel combined endoscopic approach. Surg Endosc 30:770–1, 2016.

Nirgianakis K, Papadia A, Grandi G, McKinnon B, Bolla D, Mueller MD. Laparoscopic management of ectopic pregnancies: a comparison between interstitial and 'more distal' tubal pregnancies. Arch. Gynecol. Obstet. 295:95–101, 2017.

Gasparri ML, Besharat ZM, Besharat RA, Ruscito L, **Nirgianakis K**, Farooqi AA, Papadia A, Ferretti E, Benedetti P, Mueller MD. Current knowledge of miRNAs as biomarkers in Breast Cancer. Recent Trends in Cancer Biology: Spotlight on Signaling Cascades and microRNAs. Springer 221-231, DOI: 10.1007/978-3-319-71553-7_12, 2017

Sendi P, Moser Schaub EM, **Nirgianakis K**, Hathaway LJ, Bittel P, Goldblatt D, Streit S. An Uncommon Site of Streptococcus pneumoniae Colonization Leading to Recurrent Pneumococcal Disease. Open Forum Infect Dis 4, ofw257, 2017

Papadia A, **Nirgianakis K**, Gasparri ML, Grandi G, Bolla D, Klaeser B, Mueller MD. PET/CT guided surgical excision of small abdominal wall metastases in morbidly obese endometrial cancer patients. Minerva Ginecol 69:206–207, 2017

Kocbek V, Imboden S, **Nirgianakis K**, Mueller M, McKinnon B. Dual influence of TNFα on diverse in vitro models of ovarian cancer subtypes. Heliyon. 7:e06099, 2021.

LETTERS TO THE EDITOR

Nirgianakis K, Mueller M, Kuhn A. Re: Pelvic organ function before and after laparoscopic bowel resection for rectosigmoid endometriosis: a prospective, observational study. BJOG 123:1871, 2016

Nirgianakis K, Kuhn A, Mueller M. Regarding "The Role of Transillumination in a Laparoscopic Resection of a Cesarean Scar Niche". JMIG 27:981-982, 2020

Siegenthaler F, Knabben L, Mohr S, **Nirgianakis K**, Imboden S, Mueller MD. What Is the Future of Indocyanine Green and Near-Infrared Imaging in the Surgical

Management of Endometriosis? Acta Obstet Gynecol Scand online ahead of print, 2020

Nirgianakis K, Spaanderman M, Kramer BW, Mueller M. The Potential of Glioma-Associated Oncogene Homolog 1 (GLI1) as a Therapeutic Target in Endometriosis. Ann Trans Med 8:420, 2020

Curriculum Vitae

Konstantinos Nirgianakis was born on March 19th, 1985 in Heraklion (Greece). He graduated the secondary school with honors (overall grade: 19.4/20) and entered the Department of Medicine at the National and Kapodistrian University of Athens, Greece in 2002. Konstantinos received a scholarship from the University of Athens during his studies and received his medical degree with excellence (overall grade: 8.57/10) in 2008. Following a second four-year scholarhip from the University of Athens, he initiated his residency in Germany (one year of General Surgery in Lindau and two years of Obstetrics and Gynecology in Villingen-Schwenningen). In order to continue his residency in a university hospital he moved to Bern, Switzerland and received his board certification in 2015 from the Department of Obstetrics and Gynecology at the University Hospital Bern. Since then he works as a Consultant Gynecologist and Subspecialist in Operative Gynecology and Reproductive Surgery in the same department while he gained the subspeciality certificate for operative Gynecology and Obstetrics in 2019. His research interests include the pathophysiology of pain, recurrence and treatment non-response in endometriosis/adenomyosis as well as the impact on fertility and pregnancy. Konstantinos received in September 2021 the venia docendi (Habilitation) at the University of Bern, Switzerland.