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Does presence of adenomyosis affect reproductive outcome in IVF cycles? A retrospective analysis of 973 patients

BIOGRAPHY

Dr Sunita Sharma obtained her medical training (MBBS) in 1994 and MD in Obstetrics and Gynaecology in 1996. She further specialized in Reproductive Medicine at the Institute of Reproductive Medicine, Kolkata, India, under National Board of Examinations in 2009. Presently she is working as an infertility and reproductive medicine consultant in Kolkata, India.

Sunita Sharma*, Shikha Bathwal, Nupur Agarwal, Ratna Chattopadhyay, Indranil Saha, Baidyanath Chakravarty

KEY MESSAGE

Adenomyosis adversely affects live birth rate in women undergoing IVF cycles compared with women with endometriosis who do not have adenomyosis. Screening for this entity might be considered before IVF. Affected couples should be counselled about reduced success after IVF treatment, and about the associated complications of pregnancy.

ABSTRACT

Research question: Reports on the effect of adenomyosis on assisted reproductive technology (ART) outcomes are conflicting. Does presence of adenomyosis affect reproductive outcome in IVF cycles in women pretreated with gonadotrophin releasing hormone (GnRH) agonist?

Design: In this retrospective cohort study, 973 women were divided into four groups: only endometriosis (n = 355); endometriosis and adenomyosis (n = 88); adenomyosis alone (n = 64); and tubal factor infertility as controls (n = 466). The pregnancy outcome parameters (clinical pregnancy, miscarriage rate, live birth rate) were compared between these groups.

Results: The clinical pregnancy rate was 36.62% in women with endometriosis alone, 22.72% in women with endometriosis and adenomyosis, 23.44% in women who only had adenomyosis and 34.55% in controls. Miscarriage rates were as follows: 14.62%, 35%, 40% and 13.04%, respectively. Live birth rates were 27.47% in controls; 26.48% in women with only endometriosis; 11.36% in women with endometriosis and adenomyosis; and 12.5% in women with only adenomyosis. Live birth was observed to be less in adenomyosis groups compared with controls and women with only endometriosis. No significant difference was observed in clinical pregnancy, miscarriage or live birth rate between controls and women with only endometriosis. Live birth rate was significantly different between controls and women with adenomyosis only (P = 0.01) and women with endometriosis and adenomyosis (P = 0.002).

Conclusion: Presence of adenomyosis seems to have adverse effects on IVF outcomes in clinical pregnancy rate, live birth rate and miscarriage rate. Screening for adenomyosis might be considered before ART so that the couple has better awareness of the prognosis.

Institute of Reproductive Medicine, HB-36/A/3, Salt Lake City, Sector-III, Kolkata, Kolkata 700106, India

*Corresponding author. E-mail address: sunitapalchaudhuri@yahoo.com (S. Sharma). https://doi.org/10.1016/j.rbmo.2018.09.014 1472-6483/
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KEYWORDS

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In-vitro fertilization
Live birth
Miscarriage

INTRODUCTION

Adenomyosis is a uterine pathology characterized by the invasion of endometrial glands and stroma in the myometrium leading to disruption of the uterine junctional zone. Women with adenomyosis usually have symptoms of progressive dysmenorrhoea and menorrhagia, although some cases may be asymptomatic (*Vercellini et al., 2006*). It is an important cause of sub-fertility, miscarriage and adverse perinatal outcome (*Devlieger et al., 2003; Mochimaru et al., 2014*). Transvaginal sonography (TVS) and magnetic resonance imaging (MRI) are both reliable modalities for diagnosing adenomyosis, although MRI may be marginally advantageous (*Champaneria et al., 2010*). On TVS, the pathognomonic features are asymmetrical thickening of anterior and posterior myometrium, abnormal endo-myometrial interface, heterogenous hypoechoic areas in myometrium along with anechoic myometrial lacunae. Presence of three or more sonographic features is suggestive of adenomyosis, which can be used as an appropriate screening tool (*Duelholm, 2017*).

Adenomyosis is found in a high proportion (24.4%) of infertile women, especially in those with endometriosis, those who have experienced recurrent miscarriage and recurrent implantation failure, and in older women seeking IVF treatment (*Puente et al., 2016*). Various studies have suggested that adenomyosis is not a sub-entity of endometriosis, although some symptoms may overlap between the two (*Kunz et al., 2005; Campo et al., 2012; Tosti et al., 2016*). These may be caused by different expressions of similar pathology caused by altered peristalsis in the inner myometrium (*Kunz et al., 2005*). It has been estimated that the prevalence of adenomyosis with endometriosis in infertile women is 79% and without endometriosis is 28% (*Kunz et al., 2005*). The causal association of adenomyosis with infertility has not been fully established, and its effect on natural conception and success of fertility treatment is also not clear.

The proposed mechanisms responsible for infertility and poor reproductive outcome in adenomyosis may be abnormal junctional zone myometrium leading to dysregulation of uterotubal contractility, altered endometrial function and receptivity and abnormal decidualization (*Kunz et al., 2000; Harada et al., 2016*). Inflammatory reactions mediated by prostaglandins and cytokines may also alter uterine contractions, which results in impaired utero-tubal sperm transport (*Vercellini et al., 2006*). Excessive free radical formation may deteriorate oocyte quality and embryo development in adenomyosis. It is hypothesized that free radicals also lead to activation of macrophages, T cells and increased nitric oxide exposure, resulting in abnormal implantation and early miscarriage (*Barroso et al., 1998; Ota et al., 1999; Harada et al., 2016*).

Limited studies have investigated the association between adenomyosis and assisted reproductive technology (ART) outcome. Some earlier studies have reported negative reproductive outcomes with adenomyosis (*Maubon et al., 2010; Youm et al., 2011; Ballester et al., 2012; Thalluri and Tremellen, 2012*); however, others have failed to observe such an association (*Mijatovic et al. 2010; Costello et al., 2011; Martinez-Conejero et al., 2011; Benaglia et al., 2014*). In the present retrospective study, therefore, we aimed to investigate IVF and intracytoplasmic sperm injection (ICSI) outcomes after gonadotrophin releasing hormone (GnRH) agonist downregulation in patients with ultrasonically diagnosed uterine adenomyosis.

MATERIALS AND METHODS

A retrospective cohort study was carried out at the Institute of Reproductive Medicine, Saltlake, Kolkata, India, between January 2010 and January 2015. A total of 1165 women with endometriosis, adenomyosis, or both, and tubal factor infertility undergoing their first cycle of IVF–ICSI treatment were studied. The inclusion criteria for the study were as follows: women undergoing their first cycle of IVF; Grade III and IV endometriosis confirmed by laparoscopy; adenomyosis diagnosed on two-dimensional TVS; and tubal factor infertility diagnosed on hysterosalpingography or laproscopy.

The exclusion criteria were women aged over 40 years; women with severe endometriosis who underwent excision or ablation; women with adenomyosis who underwent myolysis or wedge resection surgery; women with an endometrioma larger than 4 cm; women with fibroid, hydrosalpinx or uterine size greater than 12 weeks; and FSH greater than 12 IU/ml. Once the inclusion and exclusion criteria had been applied, 973 out of the 1165 women were selected and divided into four groups: tubal factor infertility (controls: n = 466); endometriosis alone (Group A: n = 355); endometriosis with adenomyosis (Group B: n = 88); and only adenomyosis (Group C: n = 64) (FIGURE 1). For ruling out endometriosis in the only adenomyosis group, all patients underwent laparoscopy.

All 73 patients with severe endometriosis who underwent excision or ablation were excluded for the following reasons: they were suffering from dysmenorrhoea, dyspareunia, endometrioma larger than 4 cm, or the ovary needed to be accessible before oocyte retrieval as per our clinic protocol. All women with endometriosis were diagnosed laproscopically, and all had stage III or IV endometriosis (according to revised classification of American Fertility Society) (*ASRM, 2012*). Women with severe endometriosis who underwent excision or ablation were excluded from the study. None of the women suffering from adenomyosis underwent myolysis or wedge resection surgery before IVF. In our IVF unit, baseline two-dimensional TVS within 3 months of starting IVF is routinely recommended to all women undergoing their first IVF cycle.

Patients were diagnosed with adenomyosis after visualizing at least three sonographic criteria on two-dimensional TVS, such as globular uterus caused by overall increase in myometrial thickness (n = 152); asymmetrically thickened anterior or posterior myometrial wall (n = 130); poorly defined endo-myometrial interface (n = 90); presence of heterogeneous myometrial area (n = 152); and myometrial cysts (n = 35). Asymptomatic focal adenomyosis patients (with normal size uterus) were not included in this study, as none of these patients meet the protocol for at least three sonographic criteria on two-dimensional TVS. All ultrasound scans were carried out by a single experienced sonographer to avoid inter-observer variation.

All patients had received a depot preparation of the GnRH agonist

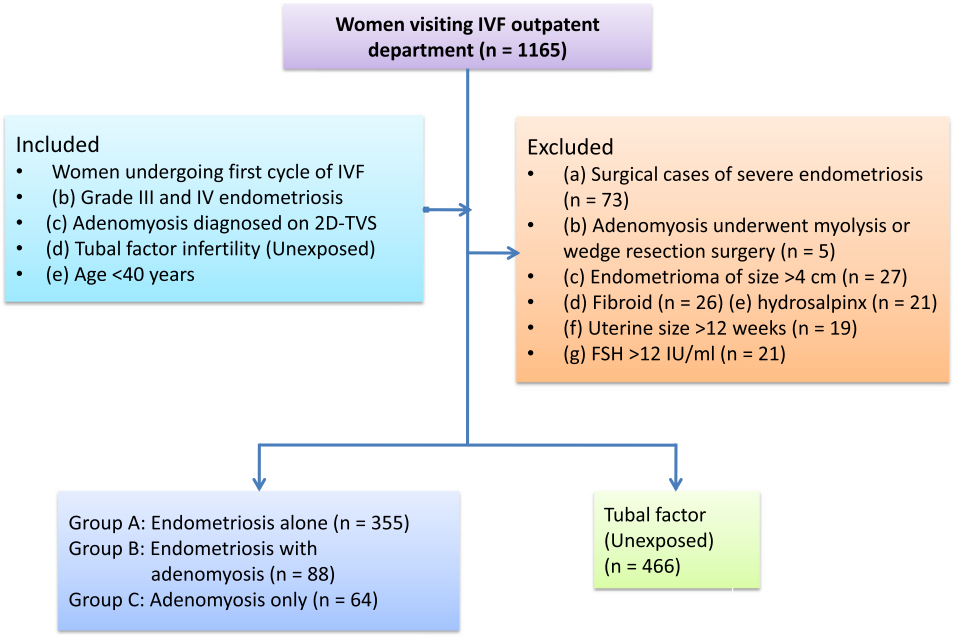

FIGURE 1 Patient recruitment in different groups after applying extensive inclusion and exclusion criteria. 2D-TVS, two-dimensional transvaginal sonography.

leuprolide acetate 3.75 mg (Lupride 4; Sun Pharmaceuticals, Mumbai, India) in three doses every 28 days. After confirmation of ovarian suppression 3 weeks after the last dose, recombinant FSH (150–300 IU; Gonal-F; Serono, Aubonne, Switzerland) was started. Ovarian folliculometry was carried out from day 6 of stimulation and then gonadotrophin dose titrated accordingly. When a minimum of two follicles reached a diameter of 17 mm or wider, HCG injection was administered (10,000 IU; Profasi; Serono, Geneva, Switzerland). Serum peak oestradiol was assessed on the day of HCG administration. Oocytes were retrieved transvaginally under ultrasound guidance 34–36 h after HCG injection. Conventional IVF–ICSI was subsequently carried out. Day 2 or 3 grade I embryos (maximum number of two) were transferred according to availability and development stage of embryos (*Veeck, 1999*). Luteal support began on the day of embryo transfer with intravaginal progesterone gel (90 mg; Crinone; Serono) daily. Serum beta-HCG level was measured 14 days after embryo transfer and, if positive, luteal support was continued up to 12 weeks of pregnancy.

Various outcome variables were assessed and analysed among all the groups. Live birth rate was taken as the primary outcome. Clinical pregnancy rate, miscarriage rate and cycle characteristics, such as gonadotrophin dose, peak oestradiol at HCG trigger, number of metaphase II oocytes retrieved, fertilization rate and number of good-quality embryos, were taken as secondary outcome measures. Clinical pregnancy was defined as presence of a viable fetus on ultrasound scan carried out 4 weeks after embryo transfer. Miscarriage rate was calculated as number of clinical pregnancies lost before 20 weeks' gestation out of total clinical pregnancies. Ongoing pregnancy is defined as viable intrauterine pregnancy of at least 12 weeks' gestation as confirmed by ultrasound. A live birth was defined as a cycle with a live fetus delivered after 26 completed weeks of gestation (as deliveries before 26 completed weeks are mostly non-viable in our scenario).

Additionally, several pregnancy complications were analysed, such as antepartum haemorrhage, preeclampsia, intrauterine growth restriction (IUGR), severe preterm delivery (non-viable delivery between 20–26 gestational weeks), intrauterine demise (IUD) and postpartum haemorrhage. After conception, women attended the institute routinely for follow-up antenatal check-ups. In the present study, 240 women delivered viable babies. Among these women, 211 delivered at our institute and the remaining 29 women delivered at different hospitals. All 29 women reported up to at least 20 weeks'gestation, and follow-up antenatal and postnatal records were provided by their respective hospitals. Complete data collection was possible for all 240 patients, and no cases were lost to follow-up.

Statistical analyses

Data analysis was carried out using statistical package Graphpad Prism 5.0. Chi squared, Fisher's exact test and Student's t-test were used for analysis, as applicable. Logistic regression models were used to calculate odds ratios of clinical pregnancy, miscarriage and live birth rates, and their 95% confidence intervals, using Medcalc. $P < 0.05$ was considered to be statistically significant.

Ethical approval

Observations encompassing this study were carried out in accordance with the Ethical Committee of the Institute (IRM/IEC/BNC-IHP/45, approved 7 February 2015). For this type of study (retrospective design), formal consent is not required.

RESULTS

Baseline and cycle characteristics were found to be comparable in all four groups (TABLE 1). No significant differences were observed in number of metaphase II oocytes retrieved, successful fertilization rate and good-quality embryos available between all

TABLE 1 BASELINE AND CYCLE CHARACTERISTICS

Parameters	Tubal (controls) (*n* = 466)	Endometriosis (group A) (*n* = 355)	Endometriosis + adenomyosis (group B) (*n* = 88)	Adenomyosis (group C) (*n* = 64)
Age (years)	33.02 ± 3.4	32.67 ± 2.53	32.12 ± 3.03	32.89 ± 2.98
Duration of infertility (years)	7.3 ± 3.2	7.6 ± 2.35	7.9 ± 3.09	8.01 ± 2.1
BMI (kg/m^2)	24.25 ± 3.02	23.72 ± 3.03	24.39 ± 3.69	24.09 ± 3.42
AFC	9.16 ± 4.28	8.6 ± 2.37	8.48 ± 1.90	9.01 ± 2.2
AMH (ng/ml)	2.89 ± 0.87	2.77 ± 0.64	2.75 ± 0.72	2.85 ± 0.79
Dose of gonadotrophins (IU)	2196.48 ± 1163.88	2390.78 ± 1148.29	2359 ± 1100.48	2232.77 ± 678.43
Oestradiol on day of HCG (pg/ml)	1270.71 ± 624.36	1370.22 ± 521.87	1287.73 ± 577	1366.16 ± 598.26
Number of MII oocytes retrieved	8.8 ± 2.6	8.44 ± 1.72	8.36 ± 1.6	8.6 ± 2.2
Fertilization rate (%)	73.52 ± 5.94	73.14 ± 5.71	72.52 ± 6.7	73.87 ± 6.29
Number of grade I/II embryos	3.6 ± 1.66	3.38 ± 1.38	3.20 ± 1.09	3.55 ± 1.31

AFC, antral follicle count; AMH, anti-Müllerian hormone; BMI, body mass index; MII, metaphase II.
Data are expressed as mean ± SD. No statistically significant differences were observed between the two groups.

the groups. The pregnancy outcome parameters are presented in TABLE 2. The clinical pregnancy rate was 34.55% in the control group, 36.62% among women with endometriosis alone, 22.72% among women with endometriosis and adenomyosis and 23.44% among women with only adenomyosis (TABLE 2). The odds ratio for clinical pregnancy rate showed that adenomyosis groups (Group B and C) had significantly lower pregnancy rate than controls and Group A (controls versus B; OR 1.79, 95% CI 1.05 to 3.06; *P* = 0.03; controls versus C; OR 1.72, 95% CI 0.93 to 3.17; *P* = 0.07; Group A versus B; OR 1.96, 95% CI 1.14 to 3.38; *P* = 0.01; Group A versus C; OR 1.89, 95% CI 1.02 to 3.50; *P* = 0.04), except when comparing controls with Group C (OR 1.72, 95% CI 0.93 to 3.17) (FIGURE 2A). The clinical pregnancy rate between controls and Group A (OR 1.03, 95% CI 0.77 to 1.37) or between Group B and C (OR 0.96, 95% CI 0.45 to 2.06,) were comparable (FIGURE 2A).

The miscarriage rate was 13.04% in the control group, 14.62% in women with only endometriosis, 35% in women with endometriosis and adenomyosis and 40% in women who only had adenomyosis (TABLE 2). It was observed to be significantly higher in women with adenomyosis compared with the controls and only endometriosis group (controls versus B: OR 0.27, 95% CI 0.09 to 0.77; *P* = 0.01; controls versus C: OR 0.22, 95% CI 0.07 to 0.69, *P* = 0.009; Group A versus B: OR: 0.32, 95% CI 0.11 to 0.90; *P* = 0.03; Group A versus C: OR 0.26, 95% CI 0.08 to 0.80; *P* = 0.02); however, it was comparable between controls and Group A (OR 0.87, 95% CI 0.44 to 1.71) and between Group B and C (OR 0.81, 95% CI 0.20 to 3.22) (FIGURE 2B).

Live birth rate was 27.47% in the control group, 26.48% in women with only endometriosis, 11.36% in women with endometriosis and adenomyosis and 12.5% in women with only adenomyosis (TABLE 2). It was also observed to be significantly lower in adenomyosis groups (Group B and Group C) compared with controls and women with only endometriosis (controls versus B: OR 2.95, 95% CI 1.48 to 5.88; *P* = 0.002; controls versus C: OR 2.65, 95% CI 1.22 to 5.71; *P* = 0.01; Group A versus B: OR 2.81, 95% CI 01.40 to 5.65; *P* = 0.004; Group A versus C: OR 2.52, 95% CI 1.16 to 5.49; *P* = 0.02) (FIGURE 2C). Similar to the trend observed for clinical pregnancy and miscarriage, live birth rate was also comparable between controls and Group A (OR 1.05, 95% CI 0.77 to 1.43) and between Group B and C (OR 0.90, 95% CI 0.33 to 2.42) (FIGURE 2C).

Pregnancy complications, such as antepartum haemorrhage, preeclampsia, IUGR, severe preterm delivery, IUD and postpartum haemorrhage are presented in TABLE 3. Non-viable severe preterm and IUD for controls, Group B and C were 12, 3 and 1, respectively; for Group A, out of 19 such cases, two severe preterm babies survived. It was observed that various pregnancy complications were marginally higher in patients with adenomyosis (Group B and C) compared with controls and Group A. The numbers, however, were too small in both groups to infer any statistically significant meaning (TABLE 3), except for IUGR, when controls were compared

TABLE 2 PREGNANCY OUTCOME PARAMETERS

Pregnancy outcome	Tubal (controls)	Endometriosis (group A)	Endometriosis + adenomyosis (group B)	Adenomyosis (group C)
Clinical pregnancy rate, *n* (%)	161/466 (34.55)	130/355 (36.62)	20/88 (22.72)	15/64 (23.44)
Miscarriage rate/pregnancy, *n* (%)	21/161 (13.04)	19/130 (14.62)	7/20 (35)	6/15 (40)
Live birth rate, *n* (%)	128/466 (27.47)	94/355 (26.48)	10/88 (11.36)	8/64 (12.5)

Non-viable severe preterm and intrauterine demise for controls; group B and C were 12, 3 and 1, respectively; for group A, out of 19 such cases, two severe preterm babies survived.

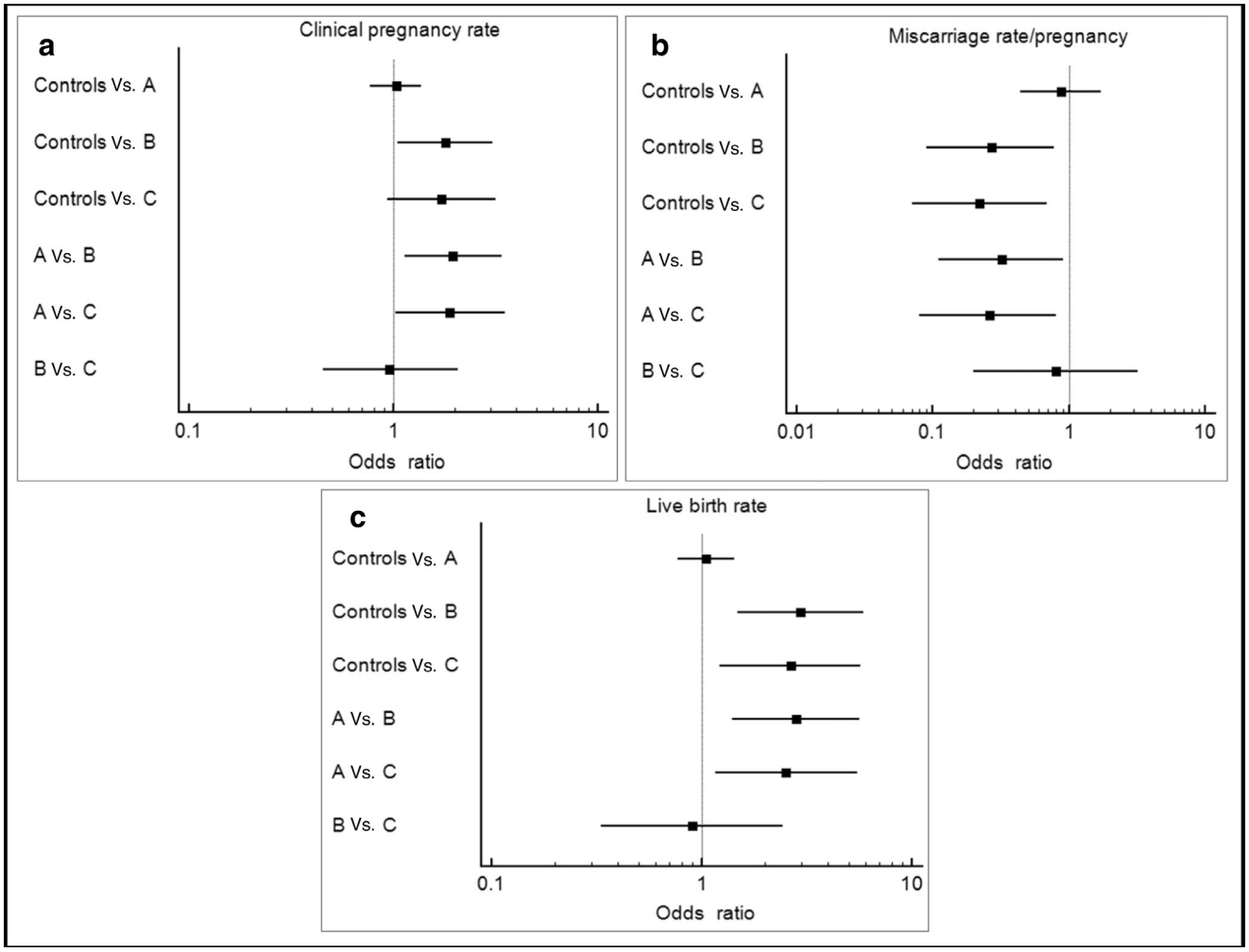

FIGURE 2 Forest plots comprising different group analysis and depicting significance of (a) clinical pregnancy rate; (b) miscarriage rate per pregnancy; and (c) live birth rate between the four groups: controls (tubal); group A (endometriosis); group B (endometriosis + adenomyosis); and group C (only adenomyosis).

TABLE 3 COMPARISON OF PREGNANCY COMPLICATIONS

	Tubal (controls)	Endometriosis (group A)	Endometriosis+ adenomyosis, and only adenomyosis (groups B + C)	Controls versus group B + C	Group A versus group B+C
PPH	13/140	11/111	2/22	OR 1.02 (95% CI 0.21 to 4.78) *P* = NS	OR 1.10 (95% CI 0.22 to 5.34 *P* = NS
APH	9/140	8/111	1/22	OR 1.10 (95% CI 0.22 to 5.34) *P* = NS	OR 1.63 (95% CI 0.19 to 13.74) *P* = NS
PET	7/140	9/111	4/22	OR 1.55 (95% CI 0.44 to 5.43) *P* = NS	OR 2.60 (95% CI 0.77 to 8.7) *P* = NS
IUGR	7/140	13/111	5/22	OR 0.17 (95% CI 0.05 to 0.62) *P* = 0.007	OR 0.45 (95% CI 0.14 to 1.42) *P* = NS
Severe preterm	9/140	14/111	3/22	OR 0.43 (95% CI 0.10 to 1.75) *P* = NS	OR 0.91 (95% CI 0.23 to 3.49) *P* = NS
IUD	3/140	5/111	1/22	OR 0.48 (95% CI 0.05 to 4.84) *P* = NS	OR 1.03 (95% CI 0.11 to 9.32) *P* = NS

APH, antepartum haemorrhage; IUD, intrauterine demise; IUGR, intrauterine growth restriction; PET, pre-eclampsia; PPH, postpartum haemorrhage.
All data are as per ongoing pregnancy, defined as viable intrauterine pregnancy of at least 12 weeks'gestation confirmed by ultrasound.
NS, non-significant *P*-values.

TABLE 4 SONOGRAPHIC CRITERIA FOR ADENOMYOSIS AND PREGNANCY OUTCOME

Sonographic criteria	Endometriosis + adenomyosis (group B)			Adenomyosis (group C)		
	Only three criteria	Only four criteria	All five criteria	Only three criteria	Only four criteria	All five criteria
Total adenomyosis patients, *n* (%)	22/88 (25)	36/88 (40.9)	30/88 (34.1)	11/64 (17.2)	24/64 (37.5)	29/64 (45.3)
Clinical pregnancy rate, *n* (%)	8/88 (9.1)	6/88 (6.8)	6/88 (6.8)	5/64 (7.8)	6/64 (9.4)	4/64 (6.3)
Miscarriage rate/ pregnancy, *n* (%)	1/20 (5)	3/20 (15)	3/20 (15)	0/15	2/15 (13.3)	4/15 (26.7)
Live birth rate, *n* (%)	4/88 (4.5)	3/88 (3.4)	3/88 (3.4)	4/64 (6.32)	2/64 (3.)	2/64 (3.1)

with Group B and C together (OR 0.17, 95% CI 0.05 to 0.62; $P = 0.007$) (TABLE 3).

The patients with adenomyosis (Groups B and C) were further classified into three sub-groups depending on the number of sonographic criteria met for diagnosis. These sub-groups were patients diagnosed with only three sonographic criteria; only four criteria; and all five criteria. Also, the representation of the patients in each of these sub-groups and their pregnancy outcome are presented in TABLE 4. Adenomyosis patients selected through higher number of sonographic criteria (five-criteria) have marginally high miscarriage rates and lower live birth rates compared with patients selected through three or four criteria. The numbers, however, were too small for any statistical significance. The characteristics of the included endometriosis groups are presented in TABLE 5.

DISCUSSION

Considering the higher incidence of adenomyosis in association with endometriosis, the need to investigate the adverse effects of adenomyosis on ART outcomes is greater. Limited studies have investigated negative reproductive outcome in women with adenomyosis; nevertheless, controversies still exist. Our group, for the first time, conducted a retrospective study in a large cohort to examine the effect of adenomyosis with and without endometriosis on ART outcomes. Our analysis suggests a negative effect of adenomyosis on IVF–ICSI outcome either alone or in the presence of endometriosis compared with women who only have endometriosis or tubal factor infertility (controls).

It is well-documented that prevalence of adenomyosis is higher among subfertile women, particularly in association with pelvic endometriosis (*Kunz et al., 2005*). In our Institute, incidence of adenomyosis alone is observed to be 13.78% in patients undergoing IVF, and presence of coexisting adenomyosis in women with endometriosis is as high as 42.11%. It was interesting to observe that mean age of the study groups was less (32.12 ± 3.03 to 33.02 ± 3.4), considering the average duration of infertility ranging from 7–8 years (TABLE 1). Relatively long durations of infertility in this study may be attributed to the early age of marriage and childbearing being common in India. Moreover, couples usually delay reporting to a tertiary care infertility clinic because of lack of proper knowledge and awareness. Economical constraint is another important factor for delaying the start of the treatment.

It is still challenging to diagnose adenomyosis, as no clear consensus has been reached on the investigation of choice. Two-dimensional and three-dimensional TVS and MRI are commonly used for diagnosis of adenomyosis owing to their similar accuracy (*Champaneria et al., 2010*). Some clinicians, however, have recommended the use of MRI over two-dimensional TVS in detecting adenomyosis owing to its improved specificity and sensitivity (*Champaneria et al., 2010*). Considering the widespread availability of ultrasonography and relatively low cost, it is the most feasible diagnostic method available for adenomyosis. In the present study, we used TVS as the diagnostic tool for adenomyosis and observed that most of the patients in the adenomyosis group fulfilled all the sonographic criteria as mentioned above. *Gordts et al. (2008)* proposed a MRI-based simple classification of adenomyosis as simple junctional zone hyperplasia; partial or diffuse adenomyosis; and

TABLE 5 CHARACTERISTICS OF ENDOMETRIOSIS IN DIFFERENT GROUPS

Characteristics of endometriosis	Endometriosis (group A; *n* = 355)	Endometriosis + adenomyosis (group B; *n* = 88)
Unilateral endometrioma, *n* (%)	139 (39.15)	31 (35.23)
Bilateral endometrioma, *n* (%)	74 (20.85)	33 (37.5)
Endometriotic nodules, *n* (%)	67 (18.87)	9 (10.23)
Endometrioma + nodules, *n* (%)	75 (21.13)	15 (17.054)
Size of endometrioma,[a] *n* (%)		
<2 cm	72 (25)	17 (21.25)
2–3 cm	130 (45.14)	42 (52.5)
3–4 cm	86 (29.86)	21 (26.23)

[a] A total of 288 patients in group A and 80 in group B had endometrioma.

adenomyoma. It is thought that a close relationship exists between the severity of adenomyosis and symptoms, implantation failure and infertility (*Gordts et al., 2008*; Naftalin 2014). Although consensus on adenomyosis classification is lacking, based on TVS observations, adenomyosis can be loosely classified into three subgroups: adenomyoma, external adenomyosis and internal adenomyosis (*Bazot et al., 2018*). In the present study, Group B and C patients were women suffering from diffuse adenomyosis having met more than three sonographic criteria (TABLE 4), whereas patients with only focal adenomyosis were excluded.

A recent meta-analysis inferred that clinical pregnancy rate in infertile women with adenomyosis undergoing IVF–ICSI was decreased by 28% (*Vercellini et al., 2014*). These findings are in agreement with our study, in which patients with adenomyosis, who either did or did not have coexisting endometriosis, had significantly lower clinical pregnancy rate than in the endometriosis group. Our findings are further supported by another group that reported higher pregnancy rate in patients with only endometriosis compared with coexisting endometriosis and adenomyosis (*Landi et al., 2008*). A multicentre study also found a significantly reduced cumulative pregnancy rate in women with endometriosis associated with adenomyosis than in the group with only endometriosis (*Ballester et al., 2012*). Presence of four or more sonographic criteria, though not statistically significant, seem to be linked to a higher miscarriage rate and lower live birth rate (TABLE 4). Therefore, patients associated with higher grades of adenomyosis are more likely to have a greater detrimental effect on reproductive outcome. This may be one of the reasons for observing comparatively poor pregnancy outcome of adenomyosis patients recruited for our study. Patients with a milder form of adenomyosis might have conceived with other methods of infertility management, thus not requiring IVF.

Our study reported a significant increase in miscarriage rate in groups associated with adenomyosis compared with groups with only endometriosis or controls, despite having similar numbers of good-quality oocytes and embryos. As with our study, a higher first-trimester miscarriage rate was also reported compared with controls in adenomyosis patients (*Costello et al., 2011; Salim et al., 2012*). Another researcher also observed a two-fold increase in miscarriage rate in patients who had all used donor oocytes (*Martınez-Conejero et al., 2011*). This indicates that chances of miscarriage are higher in adenomyosis irrespective of the quality of oocyte or embryo (*Vercellini et al., 2014*). Some researchers have suggested a possible link between uterine junctional zone abnormality and increased miscarriage rate in adenomyosis patients undergoing IVF treatment (*Chiang et al., 1999*).

Scala et al. (2017) observed that patients with diffuse adenomyosis, compared with focal adenomyosis or only endometriosis, had significantly lower mean uterine artery pulsatility index in their first two trimesters, and higher incidence of small for gestational age infants. This suggests that diffuse adenomyosis is an independent risk factor for small for gestational age infants. In the present study, along with obstetrical complications, such as pre-eclamptic toxaemia and severe preterm delivery, IUGR was observed to be significantly higher in the adenomyosis group (Group B and C) compared with controls. The numbers were also higher in Group B and C compared with the only endometriosis (Group A), although the observation was not statistically significant, possibly owing to the small number of such cases in each group (TABLE 3). Our observations are also in agreement with *Shin et al. (2018)* who reported increased risks of preterm delivery and low birth weight in patients with adenomyosis who conceived after ART treatment. The pregnancy complication arising in adenomyosis patients may be attributed to a disruption of the junctional zone, which could affect the process of the junctional zone spiral artery remodelling, defective deep placentation and placental insufficiency (*Brosens et al., 2013; Scala et al., 2017*).

Prolonged down-regulation with GnRH agonists may improve reproductive outcome in adenomyosis patients as proposed by some investigators (*Costello et al., 2011; Tremellen and Russell, 2011; Benaglia et al., 2014*). It has been suggested that long down-regulation leads to hypo-oestrogenic state continuing up to ovarian stimulation, which could have normalized the endometrial disorder present in the adenomyosis group (*Mijatovic et al., 2010*). Other studies have also found GnRH agonist to be helpful in improving pregnancy rate in patients with adenomyosis who had previously undergone multiple failed IVF cycles (*Chiang et al., 1999*). In addition to pituitary suppression, GnRH agonist was found to have direct anti-inflammatory and anti-angiogenic effect leading to apoptosis of the local disease process (*Tesone et al., 2008; Khan et al., 2010*). Down-regulation with GnRH agonists may suppress the disease process of adenomyosis but increases the dose requirement of gonadotrophins (*Mijatovic et al., 2010*). We agree that improvement of endometrial milieu should be the logical intervention to optimize pregnancy outcomes in adenomyosis. Prolonged pituitary suppression will undoubtedly affect ovarian response to stimulation and decrease the number as well as quality of oocytes retrieved (*Salim et al., 2012*). Therefore, we administered only three doses of GnRH agonists before starting IVF stimulation. In contrast to the study by *Landi et al. (2008)*, our study did not find similar pregnancy rates in adenomyosis groups compared with controls. This is possibly due to GnRH agonist, which may have had a positive effect on endometrial environment but not to the extent that it completely nullifies the effect of the disease.

The main strength of the present study is that all the four groups were analysed together, and adenomyosis was considered as a separate entity. The large sample size also helped to potentiate the strength of the study. Although adenomyosis is associated with endometriosis in most cases, the 'adenomyosis only' group in the present study comprised 64 patients, which allowed better assessment of the effect of adenomyosis *per se* on the IVF results. Moreover, endometriosis was excluded by laparoscopy in all patients in the 'adenomyosis only' group. Despite the relatively large sample size of the present study, because of its retrospective nature, it suffers from several limitations, especially involving four disparate sub-groups. Therefore, our study may suffer from potential incorporation of various bias and errors owing to inadequate clinical notes and record keeping. We applied a strong inclusion and exclusion criteria to minimize the possible effect of any such

bias. In this study, ultrasound-guided scans were used instead of MRI for the diagnosis of adenomyosis, which is operator dependent. Although MRI is considered to be more accurate than ultrasound-guided scans, in our centre, many patients could not afford MRI; therefore two-dimensional TVS was used. The ultrasound-guided scan, however, was carried out by the same experienced sonologist in all patients to maintain standardization. A large-scale, multi-centre prospective study with well-defined subject selection is necessary to associate adenomyosis with poor reproductive outcome conclusively. It would be interesting to investigate underlying molecular mechanism of poor reproductive outcomes in patients with adenomyosis. Nevertheless, despite these limitations, considerably large sample size may compensate for most of the shortcomings to a certain extent.

In conclusion, the presence of adenomyosis seems to have adverse effects on IVF outcomes in clinical pregnancy rate, live birth rate and miscarriage rate. Screening for adenomyosis needs to be considered before ART so that the couple can better understand the prognosis. Hence, counselling of women with adenomyosis before starting IVF treatment is of utmost importance. Reduced success after IVF treatment and associated complications of pregnancy should be explained to the couple. Down-regulation with GnRH agonists in higher grade of adenomyosis may not always improve IVF outcome.

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