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Intralipid infusion therapy as an adjunct treatment in women experiencing adenomyosis-related infertility

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Abstract

Background: Currently, there is some evidence that adenomyosis patients using gonadotropin-releasing hormone (GnRH) agonist long downregulation (LDR) prior to embryo transfer may improve in vitro fertilization (IVF) success rate, but not to the baseline expected success where there is no adenomyosis. Given the association between adenomyosis and an aberrant endometrial immune environment, many physicians also use prednisolone or Intralipid adjuvant treatments in combination with GnRH agonist therapy, despite neither being of proven benefit.

Objective: The purpose of this study was to investigate whether the addition of prednisolone or Intralipid immune therapy to GnRH agonist LDR improves fertility outcomes in patients with adenomyosis.

Methods: This is a retrospective cohort study of 116 consecutive adenomyosis patients who underwent their first transfer of a genetically screened euploid embryo between January 2019 and December 2020 at a private IVF clinic.

Results: There was no difference in maternal age, body mass index, number of embryo's transferred and gravidity or parity among the three treatment groups. Patients who received Intralipid had a poorer prognosis with a longer duration of infertility (4 years) and a higher number of previous embryo transfers (ETs, 5 previous ETs) compared to the comparison groups. Logistic regression analysis adjustment for all covariates revealed that LDR plus Intralipid therapy produced significantly higher live birth rates (LBRs; 60%) compared to LDR alone (40% LBR); yet, the addition of prednisolone to GnRH agonist LDR (30% LBR) provided no additional live birth benefit.

Conclusion: In this retrospective analysis, we showed Intralipid adjuvant treatment in combination with GnRH agonist therapy in adenomyosis patients undergoing IVF resulted in a LBR expected in women without adenomyosis using preimplantation genetic testing screened embryos. This benefit was not seen when using prednisolone as an adjuvant to GnRH agonist LDR. Future randomized clinical trials will be required to confirm the therapeutic benefit of Intralipid in combination with GnRH agonist therapy.

Keywords: adenomyosis, GnRH agonist, Intralipid, prednisolone, pre-implantation genetic testing

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Introduction

Uterine adenomyosis is the benign pathology characterised by the presence of endometrial glands and stroma within the myometrium associated with surrounding myometrial hypertrophy and hyperplasia. Long recognised as a cause of menorrhagia, dysmenorrhoea and miscarriage, only recently adenomyosis has been linked with recurrent in vitro fertilization (IVF) implantation failure.[1] The link was not initially apparent as

adenomyosis had previously been associated with multigravidity, not nulliparity; plus, before the advent of high-quality ultrasound and widespread use of pelvic magnetic resonance imaging (MRI), adenomyosis was only accurately diagnosed histologically following hysterectomy. However, a pivotal report in 2011 showing that viable pregnancy rates in recurrent implantation failure adenomyosis patients could be improved by the use of gonadotropin-releasing hormone (GnRH) agonist hormonal therapy confirmed that the association between adenomyosis and implantation failure was indeed causational.[2]

Today, multiple studies have now linked adenomyosis with infertility, recurrent IVF implantation failure and an increased risk of miscarriage.[3] In an attempt to ameliorate this pathology, several different therapeutic approaches have been taken. For focal disease, surgical excision of adenomyosis is a reasonable option and has been reported to improve fertility potential.[4] However, for the majority of patients experiencing diffuse adenomyosis and seeking future fertility, excision is not possible without damaging the overlying endometrium and impairing implantation potential. For this group, hormonal treatment using GnRH agonists has become the preferred option. Adenomyosis, like all forms of endometriosis, is hormonally sensitive and therefore becomes inactive under the hypoestrogenic environment of GnRH agonist long downregulation (LDR).[2] While GnRH agonist downregulation has been shown to boost implantation rates and reduce miscarriage in an IVF setting, unfortunately it does not return fertility potential to that seen in women with a normal uterus. Interestingly, even with 1–2 months of GnRH agonist therapy before the transfer of a euploid screened embryo, the rate of miscarriage in adenomyosis patients is still double than that seen in controls, suggesting that GnRH agonist hormonal therapy alone does not completely reverse adenomyosis pathology.[5] While the precise underlying cause for this residual deficit is unknown, aberrant immune pathologies have been suggested as the underlying cause.[6]

Previously, we have reported that the eutopic endometrium from women with adenomyosis is characterised by an inflammatory infiltrate consisting of elevated numbers of macrophages and natural killer (NK) cells.[7] Several other research groups have also identified elevated number of endometrial macrophages, T lymphocytes and plasma cells within the eutopic endometrium of adenomyosis patients.[8–10] In addition, others have identified elevated levels of the pro-inflammatory cytokine interleukin (IL)-6 within the adenomyotic endometrium, plus elevated pro-inflammatory (Th17) and reduced tolerogenic T regulatory (Treg) cells in the peripheral blood, all combining to suggest that enhanced immune activation in the endometrium may produce 'immune rejection' of the embryo.[11,12] As a result, a few clinicians have elected to supplement GnRH agonist treatment of adenomyosis with immunosuppressive therapy using corticosteroids such as prednisolone.[5] However, because of the potential teratogenic risk of corticosteroid therapy, other physicians have preferred to use Intralipid immunotherapy. No study to date has reported a benefit from either of these immunotherapies in the setting of adenomyosis, although there is some limited evidence from small randomized clinical trials (RCTs) suggesting that Intralipid therapy may benefit IVF implantation in general.[13]

Intralipid is a sterile lipid emulsion of polyunsaturated fatty acids derived from soy bean oil and egg yolk phospholipids. Lipid emulsions have been reported to have a variety of immune-modulatory and anti-inflammatory actions including suppression of NK cell activity,[14] with this immune suppression lasting several weeks after a single infusion.[15,16] Given this activity, plus our recent observation that Intralipid therapy boosted serum granulocyte colony-stimulating factor (G-CSF), a cytokine reported to assist successful IVF embryo implantation, our IVF unit has increasingly been using Intralipid therapy in conjunction with GnRH agonist hormonal therapy to optimise implantation in patients with adenomyosis.[17,18]

The aim of this study is to retrospectively examine whether the addition of Intralipid infusion therapy around the time of embryo transfer and early in pregnancy improves IVF live birth rates (LBRs) compared to GnRH agonist therapy, with or without prednisolone adjuvant therapy. As the incidence of adenomyosis increases with maternal age, we elected to minimise the embryonic aspects of successful IVF by only comparing outcomes for adenomyosis patients undergoing the transfer of morphologically high-quality euploid embryos in a frozen-thawed transfer cycle.

Materials and methods

Outcomes

The primary outcome of this study was LBR, defined as a live pregnancy delivered at a viable gestational age (23+ gestational weeks). Secondary outcomes include biochemical pregnancy defined as a quantitative beta human chorionic gonadotropin (hCG) >40 IU/L taken 11 days following blastocyst transfer and clinical pregnancy defined as a pregnancy seen on ultrasound scan at 7–8 weeks of gestation.

Study design

This study was a retrospective case review of all adenomyosis patients undergoing the transfer of a euploid preimplantation genetic testing for aneuploidy (PGT-A) screened embryo in a frozen-thaw embryo transfer (FET) cycle between January 2019 and December 2020 at a private IVF clinic in Adelaide, Australia. Only patients undergoing their first euploid embryo transfer in this period were included in the study.

Adenomyosis was principally diagnosed by high-quality transvaginal ultrasound using well-established criteria (heterogenous myometrium with striations, asymmetrical myometrial wall thickness, sub-endometrial cysts). The diagnosis was made by a subspeciality qualified sonologist/gynaecologist. In a small proportion of women, adenomyosis was confirmed by MRI scan.

Since our earlier publications reporting that GnRH agonist therapy improved IVF live birth outcomes, it had become standard practice within our IVF unit to perform 1 month of GnRH agonist mediated downregulation therapy to inactivate the adenomyosis before embryo transfer.[2,5] As many of our adenomyosis patients are of advanced maternal age, our standard practice was to only transfer genetically screened euploid embryos, maximising pregnancy success rates per transfer. All participants in this study had previously undergone a stimulated IVF cycle with PGT-A. This typically involved a trophoblast biopsy on day 5/6 of embryo development, followed by immediate embryo vitrification. Following amplification of the blastomere DNA, next generation sequencing was used to determine the genetic status of embryos. Only euploid embryos with less than 30% mosaic status were available for transfer. In the case of early blastocysts where the delineation between inner cell mass and trophectoderm was not adequate to allow safe biopsy, non-invasive screening of the embryonic DNA in the culture media was performed. Previous verification studies in our laboratory, as yet unreported, have shown a greater than 92% concordance between biopsy and non-invasive (NI) euploid status.

IVF treatment protocol

As previous studies had reported a compelling benefit to GnRH agonist therapy in the setting of adenomyosis, all participants in this study cohort underwent GnRH agonist therapy (goserelin 3.6 mg s/c monthly or 0.1 mg triptorelin daily) between 1 and 2 months before commencing endometrial preparation, with the duration of downregulation therapy being decided by the treating physician dependant on the severity of adenomyosis and the patient's tolerance of hypo-estrogenic symptoms. Following GnRH agonist-initiated hypo-estrogenic state, endometrial preparation for implantation was achieved using either recombinant follicle stimulating hormone (rFSH) ovulation induction followed by a hCG trigger and luteal support (1500 IU hCG days 4, 7 and 11 of luteal phase) or an artificial hormone replacement regime (oestradiol valerate 2 mg tds for a minimum of 2 weeks to achieve a minimum endometrial thickness of 7 mm, followed by vaginal progesterone – Crinone/Utrogestan). In the hormone replacement therapy (HRT) setting, luteal support was maintained until 11 weeks of gestation.

A single blastocyst transfer was standard for the majority of patients (96.6%). All embryo transfers were performed under ultrasound guidance. Biochemical evidence of implantation was assessed by serum βhCG taken 16 days following ovulation or introduction of progesterone in a HRT cycle. Clinical pregnancy outcome was assessed by a transvaginal ultrasound (GE Voluson S8, GE Healthcare, Chicago, Il, USA) between 7 and 8 weeks of gestation, with all viable pregnancies then being followed to completion. The primary outcome of interest in this study was a live birth.

Immunotherapy treatment protocol

Aside from GnRH agonist treatment, many patients also underwent immunosuppressive therapy as previous studies from our group and

others have reported endometrial inflammation in the setting of adenomyosis. The choice of immunotherapy (Intralipid, prednisolone) or no immunotherapy was decided by the treating doctor in consultation with the patient's wishes. All patients with adenomyosis were offered GnRH agonist therapy as a standard, and only those patients who refused this therapy because of patient concerns regarding hypo-estrogenic side effects received no adjuvant treatment. Intralipid therapy was generally the first-line immune adjuvant used in combination with GnRH agonist treatment, especially in those women with relative contraindications to prednisolone use (insulin resistance or diabetes, past corticosteroid side effects). However, prednisolone was used as second-line immune adjuvant in those women who were unable to access Intralipid infusions due to cost or geographical isolation.

Patients receiving Intralipid immune therapy were admitted to our day surgery and administered an intravenous infusion of 100 mL of Intralipid (Intralipid 20%, Fresenius Kabi, Hamburg, Germany) suspended in 900 mL of normal saline, given over a 2- to 3-h period.

The departmental protocol for Intralipid infusion was to administer one infusion within 24 h before the embryo transfer, then repeated 7–10 days later. This was based on our earlier publication confirming continued evidence of immune modulation over that period.[17] If the patient was confirmed to be pregnant on a day 11 serum βhCG measurement, a third and final Intralipid infusion was administered. All patients had at least two Intralipid infusions and none had four infusions.

Patients undergoing prednisolone immune therapy in conjunction with GnRH agonist treatment were administered 15 mg of prednisolone daily from day 7 of the endometrial preparation cycle, continuing until 11 weeks of gestation.

Statistical analysis

All statistical analysis and graphing were performed used using Stata 16.0 (Stata Corp, TX, USA) and GraphPad Prism 9 (GraphPad Software, CA, USA). The normal distribution of variables was assessed with Shapiro's test. Data that were normally distributed were expressed as mean ± standard deviation (SD) and were compared among treatment groups using unpaired analysis of variance test. Data with non-normal distribution were expressed as a median (interquartile range) and analysed by Kruskal–Wallis's test. Post-hoc analyses with Dunn's test were conducted to adjust for multiple comparisons. For categorical variables such as LBR, a chi-square analysis was performed. A p value less than 0.05 was considered statistically significant.

In addition, univariate logistic regression models were used to examine the univariate associations of the live birth outcome with each of potential confounding factors, including maternal age, body mass index (BMI), duration of infertility, number of previous unsuccessful embryo transfer cycles, obstetric history, endometrial preparation technique and type of therapy. A 'full' multivariate model which included all the aforementioned covariates was performed to estimate the adjusted odds ratio (OR) with 95% confidence interval (95% CI) of the LBR after controlling for confounding factors.

Results

During the study period, a total of 453 PGT screened frozen-thawed euploid embryo transfers were conducted, a 116 of these being adenomyosis patients (25.6%). In patients with adenomyosis, 40 had a FET undergoing only GnRH agonist treatment, 30 with GnRH agonist treatment and prednisolone and 35 GnRH agonist treatment plus Intralipid. A further 11 patients declined to undergo GnRH agonist or immune therapy, acting as an untreated control. This untreated control group was not considered in the final analysis of pregnancy outcomes given the objective of the study (assessment of the impact of immune adjuvant therapies) and the small size of this group. However, pregnancy outcomes in this untreated control are reported as a 'yardstick' of IVF success in adenomyosis patients not receiving GnRH agonist treatment.

The baseline demographic and clinical characteristics are reported in Table 1. Of note, there was no significant difference in maternal age, BMI, or obstetric history between the three treatment groups. However, compared with women who had GnRH agonist alone or GnRH agonist plus prednisolone, women undergoing GnRH agonist plus Intralipid therapy did have a significantly longer duration of infertility (adjusted p values

Table 1. Clinical and demographic characteristics of the study cohort.

Patient characteristics	GnRH agonist + Intralipid ($n=35$)	GnRH agonist only ($n=40$)	GnRH agonist prednisolone ($n=30$)	No adjuvant ($n=11$)	Statistical difference (p value)
Maternal age (years)	37.2 ± 3.5	34.8 ± 6.8	38.2 ± 4.9	36 ± 3.6	0.115
Maternal BMI (kg/m^2)	28.0 (22.8–33.7)	26.0 (23.1–30.8)	25.3 (22.9–29.2)	26.1 (23.4–29.3)	0.388
Gravidity	1 (1–3)	1.5 (1–2)	1 (1–3)	1 (1–1)	0.96
Parity	0 (0–1)	0 (0–1)	0 (0–1)	0 (0)	0.071
Number prior embryo transfers	5 (2–7)	1 (1–2)	2 (0–4)	1 (1–1)	0.0001
Duration of infertility	4 (3–6)	3 (1.25–5)	2 (1–4)	2 (2–5)	0.009

Data are expressed as mean ± SD or median (interquartile range), depending on the normal distribution status.
BMI, body mass index; GnRH, gonadotropin-releasing hormone; SD, standard deviation.

Table 2. Pregnancy outcomes according to treatment modality.

Clinical outcome	No adjuvant ($n=11$)	GnRH agonist only ($n=40$)	GnRH agonist prednisolone ($n=30$)	GnRH agonist + Intralipid ($n=35$)	Statistical difference (p value)
Biochemical pregnancy	5 (45%)	24 (60%)	15 (50%)	26 (74%)	$p=0.126$
Clinical pregnancy	3 (27%)	23 (58%)	14 (47%)	23 (66%)	$p=0.30$
Miscarriage	3 (27%)	8 (20%)	6 (20%)	5 (14%)	–
Live birth rate	2 (18%)	16 (40%)	9 (30%)	21 (60%)	$p=0.043$*

Multivariate logistic regression was performed adjusting for potential confounding factors such as maternal age, BMI, duration of infertility, number of prior embryo transfers, obstetric history and endometrial preparation, with significant differences in pregnancy outcomes between the three GnRH agonist groups being denoted by *.
BMI, body mass index; GnRH, gonadotropin-releasing hormone.

0.001 and 0.001) and increased number of prior unsuccessful embryo transfers (adjusted p values <0.001 and 0.0023, respectively). The vast majority (96.6%) of embryo transfers involved a single embryo, with no significant difference in the number of embryos transferred per cycle between the treatment groups ($p=0.171$). In terms of endometrial preparation after GnRH agonist treatment, artificial HRT cycles were performed in 63.3% of those women receiving prednisolone, 71.4% in the Intralipid group and 57.5% for those on GnRH agonist treatment only, with the remaining patients receiving FSH ovulation induction. There was no significant difference in terms of endometrial preparation regime between the three GnRH treatment groups ($p=0.46$).

Pregnancy outcomes are summarised in Table 2. Overall, the biochemical pregnancy rate for the three treatment groups was 61.9%, with a LBR of 43.8% per transfer. Clinical outcomes are depicted in Table 2, where it is clear that the biochemical pregnancy rate and LBR of those receiving GnRH agonist therapy plus Intralipid is numerically superior. However, overall chi-square analysis showed no statistically significant difference in biochemical ($p=0.126$) or clinical pregnancy rate ($p=0.30$) between the treatment groups, but live birth outcome was significantly associated with treatment group ($p=0.043$). This difference in LBRs likely reflects the fact that there was a twofold increased miscarriage rate seen in adenomyosis patients undergoing GnRH agonist treatment without Intralipid, compared to

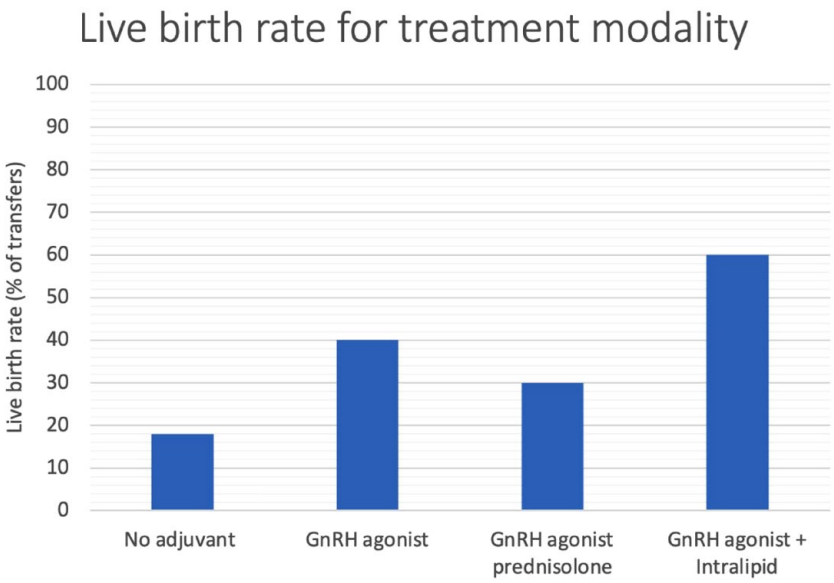

Figure 1. Live birth rate (%) according to treatment modality.

those receiving GnRH agonist and Intralipid therapy. Interestingly, while not included in our formal statistical analysis, the miscarriage rate in the small cohort not undergoing any adenomyosis treatment was particularly high (60%), resulting in a LBR of only 18.2%, a third of that seen in the GnRH Intralipid cohort (61.9%).

Univariate logistic regression suggested that there was no significant relationship between the type of treatment and live birth outcomes. However, following adjustment for all covariates (age, BMI, duration of infertility, number prior transfers, obstetric history and type of endometrial preparation), the addition of Intralipid therapy to GnRH agonist LDR did produce a significant improvement in the LBRs compared to GnRH agonist LDR alone (adjusted OR 3.101, 95% CI 1.004–9.958, $p = 0.049$). There was no significant difference in the LBRs between the LDR plus prednisolone group compared to LDR therapy alone (adjusted OR 0.531, 95% CI 0.175–1.613, $p = 0.264$) (Table 1).

All participants undergoing Intralipid therapy are monitored during the infusion and for 20 min after completion of infusion. The only side effect noted was mild irritation and erythema at the infusion site in a minority of patients.

Discussion

The use of Intralipid 'adjuvant therapy' to optimise IVF outcomes has recently come under significant criticism from both physician groups and regulators such as the HFEA (UK) and VARTA (Australia). This criticism is understandable since the link between immune dysfunction and IVF implantation failure/miscarriage is under hot debate, plus the results of Intralipid RCTs have been variable. While some RCT studies have shown Intralipid to be of benefit, others have not.[13] However, these conflicting results are not surprising given that the dose of Intralipid used varies by as much as 25-fold in these studies, plus recurrent implantation failure is caused by a heterogeneous group of pathologies, with possibly not all being amenable to Intralipid treatment.

Our study is the first of its kind to provide some level of support for the use of Intralipid therapy in combination with GnRH agonist treatment in the context of adenomyosis. While not a prospective placebo-controlled RCT, the large improvement in live birth outcome certainly suggests a beneficial impact. The observed LBR of 60% following Intralipid/GnRH agonist treatment suggests a complete normalisation of reproductive potential as this Figure 1 approximates the rates of live birth observed in good prognosis non-adenomyosis IVF

patients following the transfer of a euploid embryo.[19] Interestingly, the LBR for patients receiving prednisolone and GnRH agonist therapy was not statistically superior to those receiving GnRH agonist alone. Therefore, given the absence of any additional therapeutic benefit, plus the potential for teratogenic risk,[20] corticosteroids such as prednisolone should no longer be part of adenomyosis treatment. However, the LBR in our adenomyosis cohort that refused GnRH agonist treatment was particularly poor (18.2%), with an exceptionally high miscarriage rate given the transferred embryo was euploid. This result is identical to what we reported in an earlier study and underscores the absolute requirement for GnRH agonist treatment in adenomyosis patients undergoing IVF.[5]

Being a retrospective study, we are unable to determine the mechanism by which Intralipid may assist successful pregnancy. However, as multiple studies have identified immune dysregulation in adenomyosis patients,[7–12] plus reported Intralipids ability to modify immune responses,[15–17] the most likely therapeutic mechanism is immunological. However, the observation that generalised immune suppression with prednisolone did not offer any pregnancy benefit suggests that a hostile immune reaction 'attacking' the implanting embryo may not be responsible for poor implantation rates and higher miscarriage risk. Conversely, we would like to suggest that it may be an inadequate immune response to the implanting embryo that impairs fertility outcomes in adenomyosis patients. Previously, it has been suggested that corticosteroid immune suppression to augment IVF outcomes is a faulty premise since controlled endometrial inflammation and activation of an immune response to an embryo is actually essential for implantation.[20] Rather than attacking the embryo, endometrial NK cells, M2 immunosuppressive macrophages and regulatory T cells may instead release growth factors and cytokines which actually enhance trophoblast development and invasion. Importantly, a Cochrane meta-analysis incorporating 13 trials has concluded that there is no clear evidence that peri-implantation corticosteroid administration improves LBRs in routine IVF cycles.[21] While these trials did not target women with adenomyosis, they did target women with recurrent implantation failure, and therefore it would be expected that many trial participants did have adenomyosis and yet they saw no pregnancy benefit for corticosteroid immune suppression. As such, our finding of no benefit from corticosteroid therapy in adenomyosis is consistent with this published literature.

While some studies have provided evidence for an overactive immune system in adenomyosis, others have reported a diminished endometrial inflammatory reaction or an immunosuppressive environment. For example, those studies reporting elevated endometrial macrophage density in adenomyotic endometrium have used immunohistochemical techniques such as CD163 or CD 68 that identify 'immunosuppressive' M2 macrophages, not classical 'hostile' M1 macrophage phenotype.[7,22] While M1 macrophages release cytokines such as TNFα and IL-1 that damage and inhibit proliferation of surrounding cells, the M2 macrophages release cytokines such as TGFβ and IL-10 that promote proliferation and repair of surrounding cells.[23] Therefore, the presence of a higher density of M2 macrophages in adenomyotic endometrium may not pose a risk of immunological rejection of the embryo. Furthermore, others have reported a decrease in immunoregulatory cytokines in the peripheral blood of adenomyosis patients, possibly reflecting an immunosuppressive microenvironment within the uterus.[24,25] Monocyte chemotactic protein-1, otherwise known as CCL2, is a key cytokine that regulates migration and infiltration of monocytes, NK cells and Th2 polarised T lymphocytes into tissue. Monocyte chemoattractant protein-1 (MCP-1) expression increases in the normal endometrium from the follicular to the secretory phase, suggesting upregulation of MCP-1 may play a role in implantation and the rapid recruitment of NK cells into the endometrium in the luteal phase.[26] However, two research groups have now reported a reduction in endometrial production of MCP-1 in patients with adenomyosis which may, in turn, alter the normal migration of NK cells and Th2 T lymphocytes into the uterus to aide implantation.[26,27] As we have reported a significant increase in plasma MCP-1/CCL2 following Intralipid infusion, one potential mechanism by which Intralipid may augment implantation in adenomyosis is to normalise uterine MCP-1 levels.[17] Another is Intralipid's ability to boost serum levels of G-CSF, an embryotropic cytokine known to enhance successful implantation in IVF when administered intrauterine or subcutaneously.[17,18] However, as we did not measure endometrial or plasma cytokines

following Intralipid treatment in this study cohort, these mechanisms presently remain only hypothetical.

The strength of this study is twofold. Firstly, it is the first to present data suggesting that Intralipid therapy may benefit's LBR in adenomyosis treatment above and beyond GnRH agonist hormonal therapy. Almost every study to date has reported an increase in miscarriage risk in adenomyosis patients, even with the transfer of a euploid embryo following GnRH agonist treatment.[5,28] The low miscarriage rate and high LBR seen in our GnRH agonist/Intralipid cohort is comparable to the success rates published for the large RCTs using preimplantation genetic screening in the setting of no uterine pathology.[19] As such, it would appear that the addition of Intralipid therapy to GnRH agonist hormonal therapy totally returns reproductive potential in adenomyosis patients in line with IVF success rates experienced in patients without adenomyosis. This is an outstanding result given that the Intralipid cohort had a longer duration of infertility and a greater number of prior failed embryo transfers, making them the worst prognosis group for successful pregnancy. However, we recognise that until a RCT of Intralipid therapy in the setting of adenomyosis is performed, no definitive conclusions can be made regarding treatment efficacy. Secondly, focusing our study exclusively to patients with high morphology euploid screened embryos allowed us to remove the principal embryonic confounding factors that plague retrospective studies such as ours. This study design, plus our use of multivariate regression analysis to control for the important prognostic variables, makes it unlikely that there is any significant bias that is responsible for our results.

The principal weakness of our study is twofold. Like all retrospective studies, it is impossible to completely rule out unrecognised confounding bias that skew the results, leading to incorrect assumptions regarding therapeutic potential. Until large placebo-controlled RCTs are conducted comparing IVF outcomes in women with adenomyosis treated with GnRH agonist, with or without Intralipid therapy, we recognise that the place for Intralipid treatment will remain controversial. We hope that this study stimulates interest in the conduct of such a RCT. In the interim, given the lack of side effects and inexpensive nature of Intralipid, we advocate more clinicians consider this therapeutic approach for their patients with intractable adenomyosis IVF implantation failure. One weakness of the study is the severity of adenomyosis was not quantified. However, it is likely that the majority of subjects had moderate to severe adenomyosis as most clinicians will generally ignore very mild focal adenomyosis of questionable significance. Furthermore, data on duration of LDR were not collected and is possibly a confounding factor; however, there was no protocol to suggest patients receiving Intralipid would have longer duration of LDR than the other treatment groups. Given the retrospective design of this study, it was not possible to analyse changes in endometrial and peripheral blood immune cell populations or cytokines in response to GnRH agonist/Intralipid therapy. We believe that this type of investigational study should be conducted before embarking on a therapeutic RCT.

Conclusion

The results of this retrospective study analysing IVF pregnancy outcomes in adenomyosis patients undergoing the transfer of an euploid screened embryo underscores three key clinical messages. Firstly, while GnRH agonist hormonal treatment appears to possibly improve IVF LBRs compared to untreated patients, this treatment does not return outcomes to the baseline rate, with miscarriage rates still being twofold higher than published RCTs using PGT-A in women with normal uterine function. Secondly, the addition of prednisolone immunosuppressive therapy to GnRH agonist treatment appears to offer no advantage over GnRH agonist alone. As such this treatment should be avoided when the only indication is adenomyosis. Finally, within the limitations of a retrospective study, we have shown Intralipid treatment to be well tolerated; and it resulted in a LBR in adenomyosis patients receiving GnRH agonist co-treatment comparable to baseline PGT screened non-adenomyosis patients. While we recognise that only a RCT can prove therapeutic benefit, until such a trial is conducted, we believe that the use of Intralipid infusions should be considered in adenomyosis patients previously experiencing unsuccessful IVF treatment.

Declarations

Ethics approval and consent to participate

This research had previously received local Institutional Review Board approval (Repromed

Scientific Advisory Committee-ID Project No: 5.1) in accordance with Australian National Health and Medical Research Council (NHRMC) guidelines. Study-specific written consent was not required due to the low-risk retrospective nature of the study. However, all participants had previously given written approval to have their clinical notes reviewed for retrospective quality audit purposes.

Consent for publication

Not applicable.

Author contributions

James Henshaw: Data curation; Formal analysis; Investigation; Methodology; Project administration; Writing – original draft; Writing – review & editing.

Kelton Tremellen: Conceptualisation; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

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Competing interests

KT holds a minority stock interest in Monash IVF, the parent company of Repromed. JH has no competing interests to declare that are relevant to the content of this article.

Availability of data and materials

Not applicable.

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