PROTOCOL

Palmitoylethanolamide and polydatin effect on pain and dysmenorrhea in women scheduled for laparoscopic treatment of possible endometriosis: a double blind randomised controlled trial

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Sponsor/s: Nil

Statement of Compliance

This document is a protocol for a research project. This study will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

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STUDY SYNOPSIS

Study Name: Palmitoylethanolamide and polydatin effect on pain and dysmenorrhea in women scheduled for laparoscopic treatment of possible endometriosis: a double blind randomised controlled trial

Protocol Number: 1 Version 0.5, dated 29 September 2020

Title:	Palmitoylethanolamide and polydatin effect on pain and dysmenorrhea in women scheduled for laparoscopic treatment of possible endometriosis: a double blind randomised controlled trial
Short Title:	The efficacy of Palmitoylethanolamide and polydatin on endometriosis pain study
Design:	Prospective double blind randomized controlled trial
Study Centres:	Gynaecology Unit 2, The Royal Women's Hospital Endosurgery Units A & B, The Mercy Hospital for Women Private practices in Parkville, Box Hill, East Melbourne, Brighton East, Fitzroy, Fitzroy North and Richmond
Hospital:	The Royal Women's Hospital, Parkville, Victoria Mercy Hospital for Women, Heidelberg, Victoria Epworth Hospitals, East Melbourne & Richmond, Victoria Frances Perry House, Parkville, Victoria Cabrini Malvern, Victoria Holmesglen Private, Victoria St Vincent's Private Hospital, Victoria Sunshine Hospital Warringal Hospital Sandringham Hospital
Study Question:	Can treatment by palmitoylethanolamide/ polydatin effectively reduce endometriosis related pain and what are the cellular and molecular mechanisms modified by PEA/PLD treatment in endometriotic lesions?
Primary Objectives:	1. To compare the change in dysmenorrhoea VAS score between PEA/PLD and placebo treatment in patients with endometriosis prior to laparoscopic surgical treatment. 2. To compare the change in dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo treatment in patients with endometriosis prior to laparoscopic surgical treatment.

Secondary Objectives	1. To compare the change in dysmenorrhoea VAS score between PEA/PLD and placebo treatment at baseline and 4 months after laparoscopic surgery for endometriosis. 2. To compare the change in dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo treatment at baseline and 4 months after laparoscopic surgery for endometriosis. 3. To compare the change in dysmenorrhoea, dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo, before surgery, in patients without surgical evidence of endometriosis. 4. To compare the change in dysmenorrhoea , dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo, before surgery, and 4 months after laparoscopic surgery without evidence of endometriosis. 5. To compare the change of quality of life between PEA/PLD and placebo treatment in women with surgical evidence of endometriosis, before and after surgery. 6. To compare changes in cellular and molecular markers from endometriotic lesions, blood and peritoneal fluid samples between patients treated by PEA/PLD and placebo. 7. To correlate changes in VAS score with changes in cellular and molecular markers, in patients with endometriosis, treated by PEA/PLD and placebo. 8. To assess the side effects of PEA/PLD.
Inclusion Criteria:	• Women aged 18 – 45 with pelvic pain scheduled for laparoscopic treatment of possible endometriosis. Both women with and without previous evidence of endometriosis on ultrasound or previous surgery are eligible. • Women who agree to use any type of contraception and avoid conceiving during the 8-week treatment phase. • English speakers
Exclusion Criteria:	• Pregnancy or actively trying to conceive • Breastfeeding • Previous hysterectomy • Suspected malignancy • Inability to provide informed consent
Number of Planned Subjects:	130 patients in each arm, 260 in total
Investigational product:	palmitoylethanolamide/ polydatin 400 mg/40 mg
Safety considerations:	Worldwide, more than 800,000 patients have been treated with PEA for various chronic pain conditions. No serious side effects have been reported (36)

Statistical Methods:	Continuous variables will be summarised as means with standard deviation if normally distributed or median with interquartile range if not. Comparisons will be performed using t-tests and Mann Whitney U tests respectively. Categorical variables will be summarised as proportions and comparisons performed using Chi-squared and Fisher exact tests. P values of <0.05 will be considered significant. The impact of the PEA treatment on continuous outcome pain measures will be assessed using linear modelling adjusting for the effect of covariates including pre-treatment pain measures.
Subgroups:	• Patients treated by palmitoylethanolamide/ • polydatin 400 mg/40 mg • Patients treated by placebo • Patients with surgically proven endometriosis • Patients with surgically proven absence of endometriosis

1. Glossary of Abbreviations & Terms

Abbreviation	Description (using lay language)
PEA	Palmitoylethanolamide
PLD	Polydatin
NSAID's	Non-Steroidal Anti-Inflammatory drugs
MCs	Mast cells
VAS	visual analog scale
PPP	Persistent pelvic pain
NGF	Nerve growth factor
VEGF	Vascular endothelial growth factor
Flk1	Fetal Liver Kinase 1
NECST	The National Endometriosis Clinical and Scientific Trials
REDCap	Research Electronic Data Capture
RAFS	Revised American Fertility Society

2. Study Sites

2.1. Study Locations

Site	Address	Contact Person	Phone	Email
The Royal Women's Hospital	20 Flemington Rd, Parkville, VIC 3052, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com
Mercy Hospital for Women	163 Studley Rd, Heidelberg, VIC 3084, Australia	Dr Emma Readman	0438788854	ereadman@melbpc.org.au
Frances Perry House (Ramsay Health Care)	Grattan St and Flemington Rd, Parkville, VIC 3052, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com
Epworth Richmond	89 Bridge Road, Richmond, VIC 3121, Australia	Dr Martin Healey	0488334519	kathandmutt@bigpond.com
Epworth Freemasons	320 Victoria Parade, East Melbourne VIC 3002, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com
Cabrini Malvern	181-183 Wattletree Rd, Malven, VIC 3144, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com

Holmesglen Private	490 South Rd, Moorabbin, VIC 3189, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com
St Vincent's Private	159 Grey St, East Melbourne VIC 3002, Australia	Dr Emma Readman	0438788854	ereadman@melbpc.org.au
Warringal Hospital	216 Burgundy St Heidelberg VIC 3084, Australia	Dr Lenore Ellett	0401360974	lenore@crosbie.com.au
Sandringham Hospital	193 Bluff Rd, Sandringham VIC 3191, Australia	Dr Michal Amir	0403274870	dr.amirmichal@gmail.com
Dr Michal Amir	767 Hawthorn Rd. Brighton East VIC 3187		0403274870	dr.amirmichal@gmail.com
Dr Martin Healey	Epworth Hospital 89 Bridge Rd, Richmond VIC 3121		0488334519	kathandmutt@bigpond.com
Dr Claudia Cheng	20 Flemington Rd, Parkville VIC 3052		0409855790	claudia.cheng@gynaecarevic.com.au
Dr Charlotte Reddington	20 Flemington Rd, Parkville VIC 3052		0413296553	c.reddington@gmail.com
Dr Emma Readman	145 Victoria Parade, Fitzroy VIC 3065		0438788854	ereadman@melbpc.org.au

Dr Lenore Ellett	163 Studley Rd, Heidelberg, VIC 3084		0401360974	lenore@crosbie.com.au
Dr Samantha Mooney	163 Studley Rd, Heidelberg VIC 3084		0402923861	samantha.mooney39@gmail.com

3. Introduction/Background Information

3.1.Lay Summary

Effective medical therapies for the treatment of endometriosis related pelvic pain are limited and are often associated with side-effects.
Palmitoylethanolamide (PEA) is a food supplement that has been shown to have anti-inflammatory action.
Polydatin (PLD) is also a food supplement that has antioxidant and pain inhibiting activities.

There have been some small studies performed to assess if the combination of PEA/PLD is helpful for persistent pain associated with endometriosis. The results suggest it might have benefit, but further studies are required.

Multiple studies that assessed PEA/PLD for pain relief in various other pain conditions have not reported any significant side effects.

The aim of this study is to determine if treatment with PEA/PLD improves endometriosis associated pain.

Women who are booked for surgical treatment of possible endometriosis will be offered participation in this study. Participation will not change their surgeon's care plan. The participants will be randomised to either receive 8 weeks of PEA/PLD treatment or placebo prior to their surgery. The endometriosis will then be confirmed or excluded during the surgery. Lesions of endometriosis, endometrium, pelvic fluid and blood will be collected where possible to assess inflammatory markers.

Participants will also complete a survey including pain and quality of life assessments. This survey will be completed at recruitment, after 8 weeks of treatment (pre surgery) and at 6 months (4 months after surgery).

We will assess the change in pain scores and quality of life scores between the 2 groups to see if PEA/PLD is beneficial.

3.2.Background information

This study is one of nine projects being run as part of a program of endometriosis research recently funded by MRFF (MRF9911715 ‘Improving diagnosis and treatment of endometriosis’ $3.94 million over 5 years). The program is being run through the University of Melbourne Department of Obstetrics and Gynaecology and contains a mixture of clinical trials and laboratory-based studies. Subjects will be recruited for the research program from the Departments’ teaching hospitals (primary sites are RWH and Mercy) as well as from listed hospitals where clinicians who are part of each project undertake public and private sessions.

Core datasets for all subjects will be collected using the National Endometriosis Clinical and Scientific Trials (NECST) Network Registry. The NECST Registry is a MRFF funded initiative to create a nationally co-ordinated database containing securely held patient details, clinical history, diagnostic results, treatment details and health outcomes, for patients who have given informed consent for their details to be included, to support research into endometriosis. This national database will underpin a comprehensive national program of clinical, basic science and translational research relevant to the needs of Australians with endometriosis, consistent with the research objectives in the National Action Plan for Endometriosis. The NECST Registry is currently in final stages of developmental testing and has conditional HREC approval from Monash Health (Study Title: Establishment of the National Endometriosis Clinical and Scientific Trials (NECST) Network Registry. ERM Reference Number: 62508. Monash Health Reference: RES-20-0000-258A). The project was reviewed by the Monash Health Human Research Ethics Committee at its meeting on 07 May 2020 and was approved subject to conditions. The responses are to be reviewed outside of the Committee by the Medical Administrator and Research Support Services staff. We will not commence recruitment for our program of research until the Monash HREC approval for NECST is finalised and Site-Specific Approvals have been obtained from our participating hospitals

Subjects recruited to projects in our program will need to be consented for both NECST and the specific project(s) they are taking part in. We are not seeking HREC approval for patients to enter data into NECST as that will fall under the Monash approval. However, as part of the current HREC application we require approval to access the relevant core data for each of our subjects collected in NECST for our research program. This will allow us to use NECST as a facility to collect core data that supports our program as well as contributes to growing the National Endometriosis Registry.

In addition to the NECST core dataset, we will collect project-specific data for each of our projects. Project-specific data may be relevant to 1 or more of our projects. To assist evaluation of our projects by the Austin HREC, we have compiled a full list of all the NECST and project specific questions that we will use, accompanied by a 'road map' indicating which questions this project (and all of our other projects) will ask of its participants. Participants will only complete questions for the project(s) that they have consented to participate in.

Paperwork and data from this project will be kept in a locked room at The Royal Women’s and Mercy Hospitals. All computerized information will be kept in a

database that is password protected. Only researchers named on this project will have access. All project-specific information will be kept for a period of 7 years after the project is completed, at which time hard copy records will be shredded and computer files deleted.

Endometriosis is a common condition with recent data estimating an 11.4% lifetime risk in women of reproductive age in Australia. Symptoms include chronic pelvic pain, infertility and fatigue (1). It is an oestrogen-dependent inflammatory disease, but its etiology and pathophysiology are unclear. Frontline treatments for endometriosis include one or more of surgery, medications containing hormones and analgesia; primarily with NSAID's. Pain control with these options is often limited and associated with a relatively high rate of side effects (2).
Inflammation has been shown to have a major role in endometriosis (3). Inflammatory processes are regulated by mast cells (MCs) (4, 5, 6) which are found close to nerve fibers in endometriotic lesions (7, 8) and play a role in several normal female processes including reproduction, pregnancy, and labour (9). It has been suggested that endometrial MC function
may be altered by the local steroid microenvironment (10). Increased numbers of activated mast cells have been found in endometriotic lesions, particularly in deep infiltrating lesions proximal to nerves, suggesting that MCs may contribute to endometriosis pain by a direct effect on nerve function (11,12). Conceivably, MC activation during inflammation may contribute to pain development and hyperalgesia in endometriotic lesions (13, 14, 15). MC targeted treatment could thus prove useful for pain relief in endometriosis (16).

Palmitoylethanolamide (PEA) is a member of the N-acylethanolamine family of fatty acid amides. It is a signaling molecule which down-regulates MC activation and microglial cell function (17, 18, 19). It is a structural analog of anandamide an endogenous ligand for cannabinoid receptors CB1 and CB2 that has been reported to show a variety of cannabimimetic activities with anti-inflammatory, immunosuppressive, analgesic, neuroprotective, and antioxidant effects (20). Recently, PEA has been proposed to enhance the activity and/or inhibit the degradation of endogenous agonists of cannabinoid receptors. This would lead to a reinforcement of their actions at the level of several possible targets, which include the heat-activated transient receptor potential vanilloid type 1 (TRPV1) channels, the receptors for capsaicin; in addition, PEA activates the peroxisome proliferator-activated receptor-a (PPARa) transcription factor, thereby suggesting PPARa as a relevant molecular target for at least some of PEA's actions. An activation of PPARa and TRPV1 channels has been demonstrated in the pathogenesis of pain in sensory neurons. Therefore, treatment with PEA, by promoting a higher degree of TRPV1 desensitization, might be associated with a good analgesic effect and a lower incidence of adverse events (21).

Transpolydatin (PLD) is a precursor of resveratrol, a phytoalexin polyphenolic compound found in a variety of plants, such as grapes, berries, and peanuts, that downregulates the inflammatory response through inhibition of synthesis and release of proinflammatory mediators and MCs degranulation and modifies eicosanoid synthesis (22). In experimental models of endometriosis, Resveratrol has been shown

to bring about regression of endometriotic lesions (23). This is likely to be related to its strong anti-angiogenic and anti-inflammatory actions (24). Polydatin (PLD) is a molecule with documented antioxidant and anti-chemotactic activities. It has been given in combination with PEA in at least 2 studies for the treatment of endometriosis associated pain (25, 26), and in other studies assessing pain control of other chronic pain conditions.

A meta-analysis published in 2017, summarized all observational and RCTs treating endometriosis associated persistent pelvic pain (PPP) with the combination of PEA/PLD up to 19 July 2015 (27). Inclusion criteria were VAS/VAS like assessment before and after treatment. Out of 13 studies, only 4 were included in the final analysis (26, 28, 29, 30). Their conclusion was that the administration of micronized PEA/trans-PLD (400 mg/40 mg) twice a day for three months provided a clinically relevant improvement of chronic pelvic pain and dysmenorrhea while improving deep dyspareunia to a limited degree. No clinically relevant improvement was found for dyschezia. The total number of patients treated by PEA/PLD in all four studies was 82. Only one study (28) was a randomized double-blind study. Another study (30) was randomized open-label and the other 2 were observational.

Since July 2015, only two more studies investigating treatment of endometriosis with PEA have been published: Caruso et al (31) showed that a combination of 300 mg PEA and 300 mg alpha-lipoic acid, reduced pain in 56 patients with endometriosis after 6 months.

De Leo V et al (32) published a multi-centre study of 60 women treated by a combination of alpha-lipoic acid (a powerful natural antioxidant and enzymatic cofactor of the mitochondrial respiratory chain), PEA and myrrh (an aromatic gum-resin extracted from a tree or shrub of the genus Commiphora that has antibacterial, antifungal and anaesthetic properties). They showed a significant reduction in pain symptoms including dyspareunia, dysmenorrhea and chronic pelvic pain, with no change in the mean diameter of endometriomas.

Previous studies comparing PEA/PLD treatment with placebo in endometriosis included no more than 20 patients in each arm and were of poor quality (28).

A large study testing the effect of PEA/PLD compared to placebo on primary dysmenorrhoea, included 110 patients in each arm (220 patients in total). An improvement of pelvic pain was seen in 98.18% (95% confidence interval 97.64% to 98.60%) of cases in the PEA/PLD group vs 56.36% (95% confidence interval 48.62% to 63.81%) in the placebo group (P< .001). The combination of PEA/PLD was more effective than placebo (P < .001). Treatment was given for 10 days, from day 24 of the cycle (33).

A meta-analysis summarizing all double blind RCT of different inflammatory chronic pain conditions treated by PEA versus placebo or other analgesia, was published in 2017 (34). 10 studies looking into sciatic pain, chronic pelvic pain, temporo-mandibular joint arthritis, vestibulodynia, carpal tunnel syndrome, and molar surgery were included. 786 patients received PEA. PEA was associated with significantly greater pain reduction compared to inactive control conditions (P <

0.001). Use of placebo control, presence of blinding, allowance for concomitant treatments, and duration or dose of PEA treatment did not affect the measured efficacy of PEA.

Both PEA and polytatin are food supplements that are been sold over the counter and are widely in use for treatment of varied conditions.

The tolerability of the PEA was reviewed in the above meta-analysis (34). Adverse events reported from PEA treatment to 786 patients included only gastrointestinal upset (2), drowsiness (1), and heart palpitations (1). No significant adverse side effects were reported in other studies. Moreover, treatment was given to patients seeking fertility, after surgical diagnosis of endometriosis while trying to conceive with no concern of teratogenicity (35).

Worldwide, more than 800,000 patients have been treated with PEA, most of them in Italy and Spain, with some in The Netherlands, Germany, UK, Canada and the US, and no serious side effects that have been reported (36).

Polydatin or it's origin Reserveol, are widely used in humans because of their reported potential antitumor and anti-inflammatory properties (37). Despite the extensive research on the benefits derived from Reserveol, there was not enough research conducted to assess its harmful effects. In high doses of Reserveol (2–5 g per day) can lead to episodes of light and mild diarrhea, nausea, hypersensitivity and anal pruritus.
It appears that Reserveol needs to be administered in extremely high doses for it to elicit a significant toxic efect in vivo. it is generally well tolerated, some adverse efects including nephrotoxicity and gastrointestinal problems were reported in human subjects taking high dose (more than 1000 mg/day) (38, 39). A 450 mg/day dose of Reserveol was reported to be a safe dose for a 60-kg person (40). Overall, Reserveol is well tolerated in healthy individuals.

Interestingly, Di Paola R. et al, (41) suggested that PEA/PLD may be of use to inhibit development of endometriotic lesions in rats. In the treated group (N=10), a smaller endometriosis lesion diameter was found (as compared to controls), with improved fibrosis score and mast cell number decrease. PEA/PLD administration also decreased angiogenesis (vascular endothelial growth factor), nerve growth factor, intercellular adhesion molecule, matrix metalloproteinase 9 expression, and lymphocyte accumulation. It also reduced peroxynitrite formation, (poly-ADP) ribose polymerase activation, IkB alpha phosphorylation and nuclear factor-kB translocation to the nucleus.

In another study on rats with induced endometriosis, markers such as MCs, chymase, NGF, VEGF, and Flk1 (VEGF receptor) were shown to be significantly reduced under PEA/PLD treatment. More importantly, treatment resulted in fewer episodes of uterine pain and smaller endometriotic cysts size (42). Pain was assessed by recognizing characteristic positional behavior known to be associated with uterine pain. These results in rats suggest that PEA/PLD may act both by reducing pain and by reducing lesion size and/or activity.

The aim of this study is to assess the efficacy of PEA/PLD for pain reduction in patients with endometriosis, and to investigate the effect of PEA/PLD on lesion cellular and molecular markers including MC, chymase, NGF, VEGF, and Flk1 (VEGF receptor) expression.

This study looks at the analgesic and anti-inflammatory effects of PEA/PLD on endometriosis related pain prior to and following surgical treatment. We aim to look at the difference in inflammatory markers in the endometriotic lesions, as well as in peritoneal biopsies and fluid between PEA/PLD and placebo.

4. Study Objectives

4.1.Hypotheses

4.1.1. There will be no change in VAS scores for dysmenorrhoea, dyspareunia, dyschezia and persistent pelvic pain between baseline pain and after 8 weeks of treatment with PEA/PLD versus placebo.

4.1.2. There will be no difference in VAS scores for dysmenorrhoea, dyspareunia, dyschezia and persistent pelvic pain after 8 weeks of treatment between patients treated by PEA/PLD compared with placebo.

4.1.3. There will be no difference in VAS scores for dysmenorrhoea, dyspareunia, dyschezia and persistent pelvic pain between patients treated by PEA\PLD compared with placebo, 4 months' after surgery.

4.1.4. There will be no change in cellular and molecular markers in endometriotic lesions, blood and peritoneal fluid in patients treated by PEA\PLD compared with placebo.

4.2.Study Aims

To assess the efficacy of PEA/PLD as a potential pain reduction treatment for endometriosis related pain

4.3.Outcome Measures

Primary outcomes

4.3.1. To compare the change in dysmenorrhoea VAS score between PEA/PLD and placebo treatment in patients with endometriosis prior to laparoscopic surgical treatment.

4.3.2. To compare the change in dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo treatment in patients with endometriosis prior to laparoscopic surgical treatment.

Secondary outcomes

4.3.3. To compare the change in dysmenorrhoea VAS score between PEA/PLD and placebo treatment at baseline and 4 months after laparoscopic surgery for endometriosis.

4.3.4. To compare the change in dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo treatment at baseline and 4 months after laparoscopic surgery for endometriosis.

4.3.5. To compare the change in dysmenorrhoea, dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo, before surgery, in patients without surgical evidence of endometriosis.
4.3.6. To compare the change in dysmenorrhoea , dyspareunia, dyschezia and persistent pelvic pain VAS score between PEA/PLD and placebo, at baseline and 4 months after laparoscopic surgery without evidence of endometriosis.
4.3.7. To compare the change of quality of life between PEA/PLD and placebo treatment in women with surgical evidence of endometriosis, before surgery and again after surgery.
4.3.8. To compare changes in cellular and molecular markers from endometriotic lesions, blood and peritoneal fluid samples between patients treated by PEA/PLD and placebo.
4.3.9. To correlate changes in VAS score with changes in cellular and molecular markers, in patients with endometriosis, treated by PEA/PLD and placebo.
4.3.10. To assess the side effects of PEA/PLD.

5. Study Design

5.1.Study Type & Design & Schedule

STUDY TYPE
Multi-centre prospective double-blind placebo-controlled randomized trial

METHODS
Patients will be identified through the Gynaecology 2 Unit outpatient clinic at the RWH, the Endosurgery Gynaecology clinics at the Mercy hospital for women, and associated private practices in Victoria. Referrals and patient histories will be screened by a specialist gynaecologist, fellow trainee or research assistant prior to clinic to identify potential participants. The study will be explained to potential participants. Patients who give their consent will be blindly randomized to receive either PEA/PLD 400 mg/40 mg or placebo. Following randomization, all participants will be asked to complete a NECST questionnaire including a set of pain VAS scales, Quality of life tool (see details in Study methodology). The same questionnaire, with additional questions regarding adverse side effects and treatment compliance, will then be filled 8 weeks later (still before surgery), and 4 months' after surgery. The questionnaire will be available online and as hard copy.
Surgery will then take place as planned with surgical samples for immunohistochemistry and RNA taken only from the Royal Women's Hospital recruited patients. Samples will be collected as described in "Study methodology".
A total number of 260 patients will be recruited, 130 in each arm.

STUDY TABLE
Study Name: Palmitoylethanolamide and polydatin effect on pain and dysmenorrhea in women scheduled for laparoscopic treatment of possible endometriosis: a double blind randomised controlled trial

Assessment/Procedure	Recruitment	After 8 weeks of treatment (pre surgery)	day of surgery	4 months' After surgery
Informed Consent	x			
Baseline Questionnaire	x			
Follow up questionnaire		x		x
Laparoscopy tissue collection (endometrium, endometriosis, blood and peritoneal fluids at RWH only) and Surgeon report			x	

5.2. Standard Care and Additional to Standard Care Procedures

Standard Care Procedures	
Procedure	**Time/Visit**
Consultation to book laparoscopy	Prior to recruitment
Laparoscopy +/- treatment of endometriosis if present	As planned by treating gynaecologist

Additional to Standard Care		
Procedure	**Time/Visit**	**Dosage**
Questionnaires	At recruitment/ pre-operative	
Treatment by either PEA/PLD or placebo	8-10 weeks prior to surgery for 8 weeks	400mg/40 mg
Questionnaires	After 8 weeks of treatment (pre surgery)	
Surgery	Fresh tissue samples (endometrium, endometriosis), blood and peritoneal fluids at RWH only	
Operation questionnaire for surgeon	At operation	
Questionnaires	4 months' After surgery	

5.3. Randomisation

Block randomization will be performed using the statistical software "R", directing subjects to treatment with 400mg/40mg PEA/PLD twice daily or placebo of identical appearance. Blocks will consist of 4 subjects who consecutively enter the trial. Randomization will be stratified based on recruitment site (Royal Women's Hospital, Mercy Hospital for Women and Private Setting), with lists produced for each site. These will be held by the dispensing compounding pharmacy that will provide the PEA or placebo in identical containers directly to the patients. The subject and medical staff will be blinded to each subject's treatment.

5.4. Study methodology

All subjects will be given oral and written information about the study and informed written consent will be obtained by our Research Nurses / Assistant (see attached patient information and consent forms). After consent has been obtained, an initial questionnaire will be completed by the women. The questionnaire includes demographic details, medical background, pain questions about menstrual pain and other pelvic pain symptoms including dysmenorrhoea, deep dyspareunia, dyschesia, daily pain and quality of life assessment.

Quality of life domains will include physical and mental health.

Recruited patients will then be blindly randomized to receive either PEA/PLD 400 mg/40 mg or placebo twice daily for 8 weeks.

Treatment will be prepared by a specialist compounding pharmacy in the form of similar capsules for PEA/PLD and placebo, distributed in similar containers with their content only known to the pharmacists.

The same questionnaire (without the demographic and medical history part) will then be filled at the end of 8 weeks of treatment (still before surgery) with additional adverse drug side effects and treatment compliance assessment.

Surgery will take place within 2 weeks of finishing the study treatment.

Final questionnaire will be sent to participants 4 months' after the surgery to evaluate pain and quality of life.

Collection of tissue Samples and clinical/medical Information

Only patients operated on at the Royal Women's Hospital will be consented for tissue/ biofluid collection. Patients operated at the Mercy Hospital for Women or private hospitals will have their surgeries as planned without any other surgical intervention.

Blood: During surgery, blood samples (25-50 mls, or 1¼ -2½ tablespoons) will be drawn and processed in the laboratories at the RWH.

Endometrial Biopsy: A routine endometrial biopsy will be collected from each woman. The biopsy will be collected following collection of a clinical biopsy during hysteroscopy (endometrial biopsies will only be collected from women providing clinical consent for an endometrial biopsy as part of her planned medical procedure). Endometrial samples will be histologically assessed for cycle stage and some will be stored in RNA later (Life Technologies) for mRNA (NGS analysis) or protein expression studies or used immediately in in vitro cell culture studies.

Endometriotic lesions/cysts and peritoneal tissue: Two small samples of endometriotic lesion tissue will be taken by scissors (not diathermy) as fresh tissue and 1) stored in RNALater for RNA extraction (NGS analysis) and 2) stored in tissue media on ice for immediate in vitro cell culture studies. The formalin fixed lesions sample(s) collected as part of the routine histopathology process and diagnosis, will be accessed for research following routine histopathology assessment.

All other endometriosis lesions will be excised routinely by monopolar diathermy and submitted for routine histopathology. Paraffin embedded ectopic endometrium or peritoneum will only be recalled from pathology following completion of histopathological review (i.e. excess to pathology diagnostic requirements).

Peritoneal Fluid: a sample of peritoneal fluid (up to 10 ml) will be removed for research purposes and frozen for later use. If peritoneal fluid is not found, a saline light wash of

the pelvis will be done. This does not pose any additional risk for the patient except for extending surgical time by another 1-2 minutes. Samples will be stored at –80°C.
If no active endometriosis is seen, a small biopsy for histological assessment, immunohistochemistry and RNA analysis will be taken by scissors from one of the utero-sacral ligaments.
Surgeons will complete a dedicated operation report on surgical findings immediately after surgery, staging the endometriosis based on the revised American Society for Reproductive Medicine classification of endometriosis (rASRM).
Surgery and post-operative care will otherwise be unaffected by participation in the study.
All surgeons and study investigators will be blinded to the patient's allocation to treatment or placebo groups.
Patients will be asked to complete a repeat questionnaire at 4 months' time after surgery.
Tissue bank samples: Endometriotic lesions and endometrium will be stored in RNALater for molecular analysis and/or formalin fixed for routine pathology. Tissues will be stored in the research precinct or recalled from pathology.
Menstrual cycle stage will be determined by pathology.
All data will be collated into REDCap database, which is a secure, web-based application for building and managing complex data currently hosted at the University of Melbourne. Only coded data will be included in the RedCap database; no identifying information will be added.

Study Timeframe
Women scheduled to undergo laparoscopy for suspected endometriosis will be recruited from gynaecology outpatient clinics and prior to surgery at the Royal Women's Hospital, Melbourne (80 patients / year), the Mercy Hospital for Women (50 patients / year) and private rooms. We aim to collect approximately 130 samples per year. This number presumes average surgical volumes of laparoscopy for pelvic pain for suspected endometriosis, accounting for a 30% non-participation rate in eligible participants at each site. Recruitment should take approximately 2 years.
Data analysis and write up will take approximately 12 months.
Total 3 years after recruitment begins to complete the study.

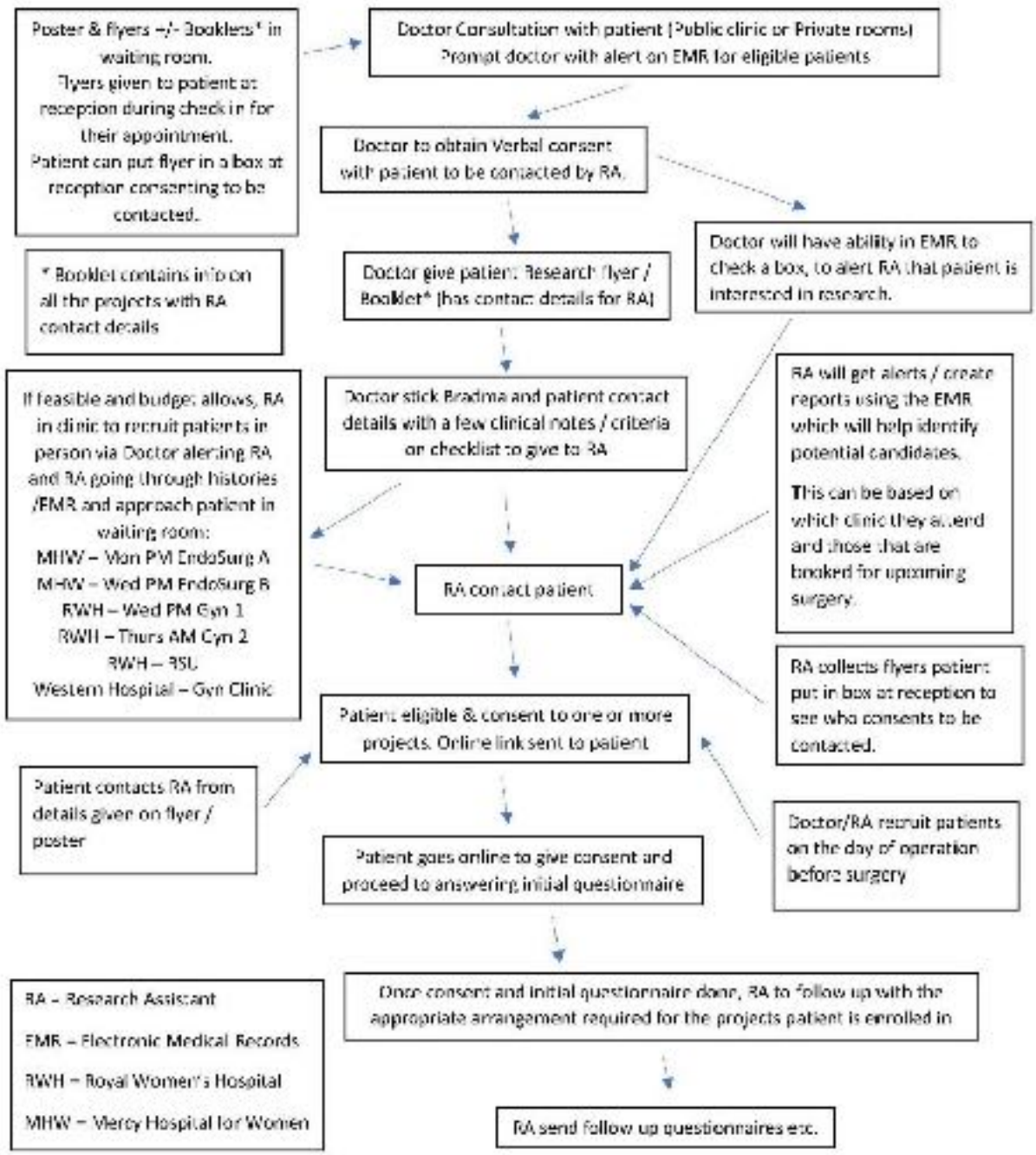
6. Study Population

6.1.Recruitment Procedure

As this clinical trial is part of a suite of projects under the umbrella of 'Improving diagnosis and treatment of endometriosis', resources for recruitment will be streamlined together. Eligible participants will be identified to be approached and recruited in a number of ways (Figure 1). Advertising poster, flyer and booklet describing all nine projects (of which this clinical trial is one) will be available in waiting rooms of participating hospital clinics and private gynaecologists' rooms. A website will also be set up through Jean Hailes for Women's Health, a national not-for-profit organisation dedicated to improving women's health.

Participants may be approached to be recruited by a gynaecologist, gynaecology trainee/resident or appointed research nurse/assistant associated with the participating unit. Eligible participants could be approached in the gynaecology outpatient clinic; surgical preadmission clinic; preoperatively on the day of surgery or preoperatively over the phone; over the phone after suitable participants are identified via medical records or if patients have provided their details on the flyer to be contacted.

Figure 1: Recruitment Procedure



6.2. Inclusion Criteria

6.2.1. women aged 18 – 45 with pelvic pain scheduled for laparoscopic treatment of possible endometriosis. Both women with and without previous evidence of endometriosis on ultrasound or previous surgery are eligible

6.2.2. women who agree to use any type of contraception and avoid conceiving during the 8-week treatment phase.

6.2.3. English speakers

6.3. Exclusion Criteria

6.3.1. Pregnancy or actively trying to conceive

6.3.2. Breastfeeding

6.3.3. Previous hysterectomy

6.3.4. Suspected malignancy

6.3.5. Inability to provide informed consent

6.4. Consent

The recruiting member of the research team will explain the study to the potential participant, answer questions and provide the participant information. Patients who agree to participate in the study will be contacted by the Research assistant over the phone to receive the consent form either in hardcopy or electronically as per the participant's preference. Consented participants will be allocated a unique trial number.

7. Participant Safety and Withdrawal

7.1. Risk Management and Safety

Safety of PEA/PLD

The tolerability of the drug was viewed in a meta-analysis of all randomized, controlled trials involving the effect of PEA on pain score of different chronic pain conditions (34). Adverse events reported with PEA treatment in 786 patients included gastrointestinal upset (2), drowsiness (1), and heart palpitations (1).

No significant adverse side effects were reported in other studies. Moreover, treatment was given to patients seeking fertility, after surgical diagnosis of endometriosis while trying to conceive with no concern of teratogenicity (35).

Worldwide, more than 800,000 patients have been treated with PEA, most of them in Italy and Spain, with some in The Netherlands, Germany, UK, Canada and the US, and no serious side effects have been reported (36)

Treatment adverse reactions will be tracked by questionnaire. In case of any adverse reaction, patient can stop treatment at any time and seek advice with the allocated contact person for complaints in each hospital or 24 hours a day at the emergency department of each hospital for severe reactions.

As the PEA/PLD supplement has not yet been definitively proven to be non-teratogenic, patients participating in the study will be requested to avoid conceiving until after treatment and surgery. Participants not using contraception will be required to undergo a pregnancy test prior to commencing the research project.

We recognize that the study treatment and questionnaires may cause distress for some participants. Therefore, wehave in placethe following actions:

1. In the PatientInformation andConsentForm (PICF)participants will be directed to contact a member of the research team (see list below of sites and contact persons) if they identify that they are experiencing distress as a result of participation in the study. The contact for Lifeline is also provided in this document.

Site	Contact Person
Epworth Hospitals	Gynaecology2 Unit Fellow (RWH)
Mercy Hospital for Women(MHW)	EndosurgeryFellow
Royal Women's Hospital(RWH)	Gynaecology2 Unit Fellow
Frances Perry House	Gynaecology 2 Unit Fellow (RWH)
Cabrini Malvern	Dr Michal Amir
Holmesglen Private	Dr Michal Amir
St Vincent's Private	Dr Emma Readman
Warringal Hospital	Dr Lenore Ellett
Sandringham Hospital	Dr Michal Amir

2. This member of the research team will identify the nature and severity of the distress and will determine further action(s) which may include:
   a. Review in thegynaecologyclinicorwiththe involvedconsultantgynaecologist
   b. Referral to psychology/psychiatry services at theMercy Hospital for Women, RoyalWomen'sHospitalor in the local community, as appropriate
   c. Linking the participant with the hospital consumer advocate
   d. Linking the patient into appropriate care at their home hospital site if not fromthe Mercy Hospital for Women orthe Royal Women's Hospital
   e. Organize acute mental health assessment by the Crisis Assessment and Treatment (CAT) team

7.2. Handling of Withdrawals

Should a participant choose to withdraw from the study no further information will be collected. If the participant withdraws prior completing 6 weeks of medical treatment, she will be removed from the study.

If the participant withdraws following the medical treatment and the second questionnaire but either prior to or after surgery, she will be asked if the data collected prior to their withdrawal is able to be used. Should they decline the data will be removed from the data bank and stored in a separate folder to ensure it is not included in the final analysis.

If the participant falls pregnant during the medical treatment phase, she will be removed from the study.

If the participant falls pregnant after the medical treatment phase and the surgery but prior the last questionnaire she will remain in the study and complete the last questionnaire as planned.

If participants have consumed less than 80% of the treatment, they will be removed from the study.

7.3. Replacements

We will continue to recruit until we have 130 participants per group for the primary outcome. Any withdrawals after this point will not be replaced because this data is for secondary outcomes only.

8. Statistical Methods

8.1. Sample Size Estimation & Justification

The sample size required for a one point difference on the numerical pain scale between PEA/PLD treatment and placebo in the change of each type of pain measured VAS score before (at recruitment) and at 4 months after surgery, and SD of 2 (based on a previous study performed by our team), was calculated to be 128 (64 in each group), based on an alpha of 0.05, power of 0.8 and two sided p-value. Allowing for a drop-out rate of 30%, and assuming that 30% of patients will not be found to have endometriosis on surgery, a total sample size required is 130 in each group (N=260).

If the participant withdraws prior to completing 6 weeks of medical treatment, she will be removed from the study.

8.2. Statistical Methods to Be Undertaken

Continuous variables will be explored with summary statistics (mean, standard deviation, quartiles, range) and graphs (box plots, dot plots). Comparisons between the PEA treatment and placebo will be performed using t-tests, or Mann-Whitney U tests if appropriate.

The impact of the treatment on continuous pain measures will be assessed using linear models adjusting for the effect of covariates, including pre-treatment pain measures.

Categorical variables will be summarized as proportions and cross tabulated frequencies, and associations examined using chi-squared and Fisher's exact tests. A modified intention to treat approach to analysis will be undertaken.

9. Storage of Blood and Tissue Samples

9.1. Details of where samples will be stored, and the type of consent for future use of samples

All samples will be processed and stored within the research laboratory at the Melbourne University, Department of Obstetrics and Gynaecology, Level 7, at the Royal Women's Hospital. Samples will be deidentified and labelled with a unique tissue bank number linked to all samples collected for each patient (blood, peritoneal fluid, endometriosis lesion, endometrium and clinical data). RNA samples will be stored long term at –80°C and histology samples will be stored long term in histology block drawers. The laboratory has key card access which restricts access to research personnel only. The laboratory is also located within the Research Precinct of RWH which also has key card access further restricting access to Departmental staff only. These two measures ensure the protection of the sample storage and all data collected relating to the patient.

10. Data Security & Handling

10.1. Details of where records will be kept & How long will they be stored

Paperwork from the project will be kept in a locked room at The Royal Women's Hospital and all computerized information will be kept in a database that is password protected. Only members of the research team will have the password. All information will be kept for a period of 7 years after the project is completed, at which time hard copy records will be shredded and computer files deleted.

10.2. Confidentiality and Security

Each subject will be provided with a unique study identification number (study ID). Identifiable information that is collected in this study will only be accessible by research staff with security access.

10.3. Ancillary data

Not applicable

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