CLINICAL STUDY REPORT

The effect of progestogens on the cardiovascular health of women with premature ovarian failure.

Trial long title A randomised controlled trial, to compare the effect of, micronized progesterone and Medroxyprogesterone Acetate on vascular elasticity, lipid profiles and the coagulation cascade of women with premature ovarian failure.

Sponsor Protocol Code:	POF01
EudraCT Number:	2012-004511-30
ClinicalTrials.gov Identifier:	n/a
REC Number:	12/LO/1957
Investigational Drugs (IMPs):	Utrogestan and Medroxyprogesterone Acetate, (Provera)
Indication:	Premature Ovarian Failure (POF)
Development Phase:	IV
Study Begin (FPFV):	29.04.2013
Study End (LPLV):	23.09.2016
Report Version & Issue Date:	Version 1 24Apr2019
Sponsor Name and Address:	King's College Hospital NHS Foundation Trust Denmark Hill London SE5 9RS
Co-sponsor contact details:	Helen Critchley King's Health Partners Clinical Trial Office F16, Tower Wing, Guy's Hospital, Great Maze Pond London SE1 9RT
Chief Investigator:	Mr Haitham Hamoda

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Ethics

Independent Ethics Committee or Institutional Review Board
The study protocol and amendments were reviewed and approved by a National Research Ethics Service London-West London.

Ethical conduct of the study
The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA)

Subject information and consent
Participants were recruited from King's College London NHS Foundation Trust over a 30-month period between 2013 and 2016.

Data Monitoring

No data monitoring committee was in place for this trial.

Sponsors, Investigators and Trial Sites

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Study Synopsis

Title of clinical trial	Trial long title A randomised controlled trial, to compare the effect of, micronized progesterone and Medroxyprogesterone Acetate on vascular elasticity, lipid profiles and the coagulation cascade of women with premature ovarian failure.
Protocol Short Title/Acronym	The effect of progestogens on the cardiovascular health of women with premature ovarian failure.
Study Phase	IV
Sponsor name	King's College Hospital NHS Foundation Trust
Chief Investigator	Mr Haitham Hamoda
Eudract number	2012-004511-30
REC number	12/LO/1957
IRAS project ID:	103936
Medical condition or disease under investigation	Premature ovarian failure
Purpose of clinical trial	To compare the effect of micronized progesterone and Medroxyprogesterone Acetate on the cardiovascular health of women with POF.
Primary objective	To assess the effect of micronized progesterone and Medroxyprogesterone Acetate on vascular elasticity. This will be assessed by examining the changes in pulse wave analysis in women with POF receiving combined oestrogen/progestogen HRT.
Secondary objective (s)	To assess the effect of micronized progesterone and Medroxyprogesterone Acetate on: 1. Pulse wave velocity. 2. Lipid profiles. 3. Coagulation cascade. 4. Side-effect profile and women's satisfaction with their HRT regimen.
Trial Design	Randomised, controlled, open label, 2-arm trial, comparing the effects of the micronized progesterone, Utrogestan®, to Medroxyprogesterone Acetate, both used in combination with transdermal oestradiol patches (Evorel®).
Endpoints	Primary endpoint will be the mean vascular elasticity change.
Planned number of subjects	90
Summary of eligibility criteria	1. Females aged between 18 and up to 45 years. 2. Confirmed diagnosis of POF with a history of oligomenorrhoea/amenorrhoea for at least 4 months and elevated gonadotrophins ≥ 30mIU/ml on two separate occasions. 3. Willingness to participate.

	4. No concomitant co-morbidities that would contraindicate the use of hormonal preparations such as unexplained bleeding, breast cancer, endometrial cancer or thromboembolic disease.
IMP, dosage and route of administration	Utrogestan, oral, 200 mg/24 hours from days 15-26 of a 28 days cycle. Medroxyprogesterone Acetate, Provera, oral, 10 mg/24 hours from days 16-26/cycle.
Active comparator product(s)	n/a
Maximum duration of treatment of a subject	12 months
Version and date of protocol amendments	Protocol version 1.0 19-Oct-2012 There have been no substantial amendments to the MHRA for this trial.
Publications	Menopause Int. 2013 Sep;19(3):127-32. doi: 10.1177/1754045313503635.

Study period (years)

Participants were recruited from King's College London NHS Foundation Trust over a 30-month period between 2013 and 2016. The date of the first patient visit (FPFV) 29.04.2013 and the last patient last visit (LPLV) was 23.09.2016.

Patient recruitment was completed in 31.07.2015. There were no interruptions (temporary halts) and whether the trial was not terminated prematurely.

Objectives

Primary Objective

To assess the effect of micronized progesterone and Medroxyprogesterone Acetate on vascular elasticity. This will be assessed by examining the changes in pulse wave analysis in women with premature ovarian failure receiving combined oestrogen/progestogen HRT.

Secondary Objective

To assess the effect of micronized progesterone and Medroxyprogesterone Acetate on:

1. Pulse wave velocity.
2. Lipid profiles.
3. Coagulation cascade.
4. Side-effect profile and women's satisfaction with their HRT regimen.

Background and Rationale

Hormonal therapy is used for a number of indications, including regulation of menstrual irregularities,

management of dysmenorrhoea, and prevention of vulvovaginal atrophy, osteoporosis and fractures in postmenopausal women [6], suppression of vasomotor symptoms, reduction in the risk of dementia and colorectal cancer [6]. The main benefit of oestrogen use is to counteract the effects of the long term sequelae of an early menopause which has been well documented. It is thought that the risk of mortality from ischaemic heart disease can be increased by 80% in these women [15]. The long term use of oestrogens is thought to lower the risk of mortality by 40% from any cause, but primarily explained by the reduction in the number of deaths seen secondary to cardiovascular disease [16].

The cardioprotective role of oestrogens is thought to work through their favourable impact on surrogate markers of cardiovascular disease such as lipids and lipoprotein profiles [17-18] (reduce low-density-lipoprotein levels [LDL] and increase high-density-lipoprotein levels [HDL]) [16, 19], endothelial function [12] and their anti-inflammatory and anti-oxidant properties [14, 17, 20]. Oestrogen replacement therapy has been shown to improve insulin sensitivity, lower diastolic blood pressure and stimulate the production of vasodilating factors such as nitric oxide [19, 21-22] and prostaglandins by the vessels [19].

However, few women are prescribed oestrogen only replacement therapy and the majority are prescribed a combination of oestrogen and progesterone. Research has suggested that the progestin component of HRT is responsible for some of the adverse effects of the drug regimen, including, coronary heart disease and breast cancer, through their androgenic and glucocorticoid properties. Despite this, the progesterone component is essential for at least 10 to 14 days per month in women with an intact uterus to reduce the risk of endometrial cancer [16, 23] (relative risk 2.1 to 5.7) [16] by opposing the proliferative effects of oestrogens [19, 24]. The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial looked at the effects of oestrogen or oestrogen/progestin regimens (conjugated equine oestrogen 0.625 mg daily, alone or in combination with one of the three regimens of progestational agents: medroxyprogesterone acetate at 2.5 mg daily, medroxyprogesterone acetate at 10 mg days 1 to 12, and micronized progesterone at 200 mg days 1 to 12) on heart disease risk factors in postmenopausal women aged between 45-64 years. It showed that unopposed oestrogen produced the most beneficial effects, but the high rate of endometrial hyperplasia restricted its use to women without a uterus. For women with a uterus, the cyclic use of a progestin produced the most favourable effects [25-26].

Randomised controlled trials including the Women's Health Initiative (WHI) Study [27], have raised widespread controversy regarding the use of progestins in HRT [22], such as medroxyprogesterone acetate, norethisterone acetate and levonorgestrel in combination with conjugated equine oestrogens or oral oestradiol [28], increasing the risk of coronary heart disease, breast cancer, pulmonary embolism and stroke [29]. However, it is important to acknowledge that these trials recruited older post-menopausal women (50-79 years of age in the WHI Study) and may have used non-physiological hormonal preparations, thus the data may not be correctly extrapolated to younger women who have undergone an early menopause [20]. Subsequent reports from the same investigators and observational studies have shown a reduction in the incidence of coronary heart disease with HRT if commenced below the age of 60, suggesting, a beneficial role in the prevention of primary disease but not in women with established coronary damage [30] as reflected by the Heart and Estrogen/progestin Replacement Study (HERS) which looked at the efficacy and safety of oestrogen plus progestin therapy (0.625 mg conjugated oestrogen plus 2.5 mg medroxyprogesterone acetate daily or an identical placebo) on the prevention of recurrent coronary heart disease events in women with established disease, aged between 44 to 79 years [31]. The Kronos Early Estrogen Protection Study (KEEPS) which is looking at women aged between 42-58 years of age, using regimes consisting of 0.45 mg of oral oestrogen (Premarin®) and a transdermal (Climara®) skin patch as well as a progesterone (Prometrium®) for the first 12 days of the month [32], and the ongoing Early Versus Late Intervention Trial with Estradiol which is looking at the effects of oral 17□-estradiol on the

progression of early (subclinical) atherosclerosis and cognitive decline in healthy postmenopausal women [33], are aiming to assess this in further detail.

Rosano et al. [25] have shown that different combinations of oestrogens-progestin can have varied effects on the vascular system and that the type of progestin used is of paramount importance. The type of progestin used can also have a varied effect on the lipid profile which is an important factor in cardiovascular disease [34]. Synthetic progestins, norethisterone and medroxyprogesterone acetate, have been shown to be associated with metabolic and vascular side effects, such as suppression of the vasodilating effect of oestrogens, in both experimental and human controlled studies [23].

Furthermore, the mode of administration may impact on the vascular properties of hormonal treatment. Oestrogen patches lead to lower peak plasma concentrations with a more sustained release of circulating oestrogen resembling physiological levels and a lower hepatic exposure, in contrast to oral preparations [13, 20]. In addition, higher doses are required orally secondary to extensive first-pass hepatic metabolism, which may consequently activate the renin-angiotensin system and thus, lead to increases in systemic blood pressure [13].

Progesterone

The biological actions of natural progesterone are multi-fold, including, antigonadotropic (inhibition of ovulation, preparation of the endometrium for implantation of a fertilised ovum), antioestrogenic (prevention of uterine contractions), antiandrogenic (prevention of the conversion of testosterone into its active metabolite dihydrotestosterone by competing with 5α-reductase) and antimineralocorticoid properties (promotion of the excretion of sodium and water) [21, 35].

There are many different classes of progestogens, each with different pharmacological properties dependent upon the parent molecule from which they are derived, testosterone or progesterone, (table 1), and thus, they have different side effect profiles [19, 35]. These differences may help to explain why progestogens can partially oppose the beneficial effects of the oestrogens [19].

Table 1. Progestogens (Modification of the 'Classification of older and new progestins' from Sitruk-Ware (2004) and 'Pharmacological profile of progestins' from Sitruk-Ware (2004).

Derived from Testosterone:
- 19-Nortestosterone derivatives *(exert some androgenic activity)*
  - Estrane group:
    - Norethisterone and its metabolites
    - Norethynodrel *(first progestin synthesized)*
  - Estrange/Pregnane group:
    - Dienogest *(significant antiandrogenic activity)*
  - Gonane group:
    - Norgestrel
    - Levonorgestrel and its derivatives:
      - Desogestrel and its derivatives
        - 3-Ketodesogestrel □ etonogestrel
      - Gestodene
      - Norgestimate □ norelgestromine
  - Spirolactone derivative
    - Drospirenone *(potent anti-mineralocorticoid progestin that exerts some anti-androgenic action too)*

Derived from Progesterone:

- Dydrogesterone
- Retroprogesterone
- 17-OH progesterone derivatives:
  - Chlormadinone acetate
  - Cyproterone acetate *(potent anti-androgen)*
  - Medroxyprogesterone acetate *(slight androgenic activity and exerts glucocorticoidic activity when given at high doses)*
  - Megestrol acetate (*50% less glucocorticoid activity than medroxyprogesterone acetate)*
- 19-norprogesterone derivatives *(do not possess androgenic, oestrogenic or glucocorticoidic activity at therapeutic doses – pure progestational molecules as they bind more selectively to the progesterone receptor)*:
  - Demegestone
  - Promegestone
  - Nestorone *(one of the most potent progestins but not active orally [35])*
  - Nomegestrol acetate
  - Trimegestone *(more potent than Nestorone)*

The effects of progesterone are mainly mediated by a progesterone intracellular receptor, located in the nucleus of target cells. Some synthetic progestogens also have an affinity for other steroid hormone receptors, such as the androgen, mineralocorticoid, glucocorticoid and/or oestrogen receptors [19, 35]. Their interaction with these receptors may be in an agonist or antagonist capacity and with differing potency, resulting in actions that differ from the effect they have on the progesterone receptors [35] (table 2 and table 3). Their potency is also determined by the dose, duration and route of administration, for example, Nestorone is more potent than progesterone when given subcutaneously, in contrast, Norethisterone is more potent when taken orally [21].

Table 2. Relative binding affinities of some progestins, expressed in percent and compared with 100% binding for the native hormone to its target receptor (from the 'Pharmacological profile of progestins' from Sitruk-Ware (2004).

Binding of progestins with human steroid receptors in vitro					
Receptor	Relative binding affinity (%)				
	TMG	MPA	NET	GES	LNG
Progesterone	588	298	134	864	323
Androgen	2.4	36	55	71	58
Glucocorticoid	13	58	104	38	7.5
Mineralocorticoid	42	3.1	2.7	97	17
Estrogen	<0.02	<0.02	0.15	<0.02	<0.02

Abbreviations: TMG: trimegestone; MPA: medroxyprogesterone acetate; NET: norethisterone; GES: gestodene; LNG: levonorgestrel; adapted from [Philibert D et al., 'The pharmacological profile of a novel norpregnane progestin (trimegestone)'. Gynaecological Endocrinology 1999;13(5):316–26].

Table 3. Biological activities of progestins and their interaction with different steroid receptors other than the progesterone receptor (taken from 'Different cardiovascular effects of progestins according to structure and activity', Nath et al. (2009).

Progestogens	Androgenic	Antiandrogenic	Glucocorticoid	Antimineralocorticoid
Progesterone	-	±	+	+
Older progestins				
MPA	±	-	±	-
NET	+	-	-	-
LNG	+	-	±	-
Newer progestins				
Dienogest	-	+	-	-
Drospirenone	-	+	-	++
Nomegestrol acetate	-	±	-	-
Trimegestone	-	±	-	±
Nestorone	-	-	-	-

+ effective; ± weakly effective; - not effective

Acne is more commonly reported with progestogens with a high degree of androgenic activity, such as levonorgestrel. Proestogens with antiandrogenic activity such as cyproterone acetate may in contrast, be used for the treatment of acne. Headaches can occur with either antigonadotropic agents or secondary to hyperoestrogenism where the oestrogen component dominates. Bloating and weight gain occur with progestogens that exhibit glucocorticoid like activity such as medroxyprogesterone acetate [35]. The anti-mineralocorticoid actions of the progestogens such as drospirenone, can antagonise the water and sodium retention effect of oestrogens and may account for their ability to lower the blood pressure of some women [19, 21-22, 28].

Furthermore, the combination of the progestogen with an oestrogen can modulate the side effect profile, dependent on the type as well as the dose of the oestrogen used [35].

Mode of administration of HRT

Many different modalities of administration of HRT are available for use, including, oral, transdermal, intrauterine and implants. Choice of preparation is both prescriber and user dependent and is greatly influenced by convenience and acceptability [36]. The mode of administration as well as the progestogens' affinity for the different types of steroid receptors can also impact on their side effect profile. For example, glucose intolerance and hyperinsulinaemia are well known risk factors for cardiovascular disease [19], and a study by Godsland et al., showed that combined HRT administered orally had a detrimental effect on glucose tolerance and resulted in an increase in plasma insulin levels when compared with transdermal therapy, despite both regimes containing a progestogen with androgenic properties [19, 37].

However, oral administration of oestrogen has been shown to have a greater impact on lipid profiles [38-39] compared to the transdermal and implant route of administration [38]. Furthermore, HDL levels and plasma triglyceride concentrations increase and Lp(a) levels (high levels of which have been associated with arterial disease) decrease with oestrogen administered orally [38]. These effects are thought to reflect the effect of the hepatic first-pass effect [38-39], as implants have been shown to produce equally high concentrations of the hormones [38]. Furthermore, the study by Seed et al. [38], showed a reduction in systolic blood pressure when HRT was administered transdermally compared with oral HRT.

Transdermal oestradiol is also not thought to affect the coagulation cascade unlike oral oestrogen, and therefore, be of less venous thrombotic and stroke risk. Experimental data suggests that transdermal oestradiol stabilises atherosclerotic plaques by reducing the concentration of inflammatory markers as well improving endothelium-dependent vasodilation in the brachial arteries

[47].

In addition, the transdermal route of administration is thought to produce more physiological levels, secondary to its slower rate of absorption and avoidance of gastrointestinal conversion of oestrogen metabolites [39], with more steady plasma concentrations [29].

Route of administration can also influence the magnitude and time course post the commencement of treatment that, changes in lipoprotein levels would be observed. For example, the oral route can produce immediate changes, whereas, the percutaneous route can take up to 6 months before the peak hormone concentrations are reached [39].

Cardiovascular disease risk factors and progesterone

The ability of synthetic progestogens to co-interact with a number of different steroid receptors, can negatively impact on cardiovascular risk factors, through their effects on the lipid profile, vasomotion [19] and carbohydrate metabolism [21]. The androgenic properties of some of the progestogens may slightly oppose the HDL-raising effect of oestrogens [19, 21, 40], as well as increase insulin resistance and impair glucose tolerance [16, 22, 28]. However, the cardioprotective role of oestrogen through its effect on the lipid profile can only account for up to 30-50% of its cardioprotective effects [16, 19]. The data in Table 4 were obtained from the review by Nath et al. [22] and depicts the action of oestrogen/progestogens on the surrogate markers of cardiovascular disease risk factors.

Table 4. Surrogate markers of cardiovascular disease risk factors and the action of oestrogen/progestogens.

Surrogate markers improved by estrogen	Role of progestins when combined with estrogen, according to their other activities		
	Antimineralocorticoid	Androgenic	Non-androgenic
Insulin sensitivity	N	-	+
Fasting glucose	N	-	+
Blood pressure	+	N	N
Lipid metabolism	+	-	+
Vasodilating factors (↑NO)	+	-	+
VSMC and collagen	N	-	+

+, positive, beneficial action; -, negative effect; N, neutral; NO, nitric oxide; VSMC, vascular smooth muscle

Micronized progesterone in contrast, is thought to reduce the incidence of new onset diabtetes when combined with transdermal oestrogen, and have a neutral or beneficial effect on blood pressure in postmenopausal women [47].

Serum lipids and lipoprotein profiles and progesterone

The main plasma lipids are cholesterol and triglycerides and they are transported by plasma lipoproteins (chylomicrons, very-low-density lipoproteins (VLDL), LDL, HDL, IDL) [41]. Of these, the serum level of LDL has been shown to have a close relation to development of coronary artery disease [21, 41]. In contrast, HDL, particularly, the second subfraction of HDL (HDL2) is thought to be inversely related to the development of atherosclerosis [41-42].

Studies have shown that progestogens used alone can lower plasma lipid levels (HDL and phospholipids) but the mechanism of action is debated as the amount of data available is limited and

to a certain degree conflicting. Those that claim a triglyceride lowering effect of progesterones, postulate whether this is secondary to their antagonistic effect on oestrogen stimulated hepatic triglyceride synthesis or due to increased activity of the lipoprotein lipase enzyme [41]. Furthermore, it has been found that the effect of progesterone on triglyceride synthesis is not seen in women who have had a bilateral oophorectomy, either secondary to their synergistic effect with other ovarian steroids or the antagonistic effect of high pituitary gonadotrophin levels [41].

The effect of progestogens on serum lipid levels can be altered by the addition of an oestrogen as well as the dose of either component [39, 41]. For example, a study by Lobo et al. [16], showed that the concomitant use of a medroxyprogesterone acetate with a conjugated oestrogen attenuated the beneficial increase in HDL cholesterol levels noted with oestrogen only treatment but did not impact on the decreases in LDL cholesterol and Apolipoprotein B levels. A further study [40], confirmed similar findings, whereby they showed a 20% increase in HDL2 cholesterol and phospholipid concentrations and a 25% decrease in activity of hepatic lipase enzyme in postmenopausal women using oestradiol valerate only. However, by adding a progestogen, levonorgestrel, the effects of oestrogen were reversed. The study by Ottosson et al. [42], also showed comparable results whereby, levonorgestrel and medroxyprogesterone acetate would lower HDL levels, particularly HDL2, the former to a greater extent, when added to oestrogen.

Overall, progestogens are thought to be able to modify the effects of oestrogen-induced changes on lipid profiles [41]. Those with the greatest effect are thought to be those with androgenic (i.e. levonorgestrel) and/or anti-oestrogenic properties [41-42]. In contrast however, oral micronized progesterone is not thought to negatively impact on the oestrogenic benefits on lipoprotein profiles [39, 42].

Haemostasis and progesterone

The menopause is associated with an increased prothrombotic state associated with changes in clotting factors such as fibrinogen and factor VII [22]. Furthermore, the use of hormonal therapy has been shown to increase the relative risk of venous thromboembolic disease by two- to four-fold [43, 48]. The WHI Study showed that there was a two-fold greater risk of developing a VTE in those using combined oestrogen and progesterone compared to placebo [27]. The HERS and Estrogen Replacement and Atherosclerosis trial (which looked at the effects of oestrogen replacement therapy [0.625 mg/day oral conjugated equine oestrgen] with or without continuous low-dose progestogen [2.5 mg oral medroxyprogesterone acetate/day] versus placebo on the progression of atherosclerosis) found a 1.7% and 2.6% respective increase in VTE events in those taking HRT [43].

The risk of developing a VTE varies depending on the preparation used, oral versus transdermal, as well as the presence of other risk factors which can be additive [43]. The ESTHER (EStrogen and THromboEmbolism Risk) study group, a French case-controlled study, reported that oral but not trandermal oestrogen is associated with an increased risk of VTE in postmenopausal women [44, 47]. Transdermal oestradiol is not thought to increase the risk of VTE more than that of non-users [47]. This is thought to be secondary to its mechanism of metabolism [48]. Transdermal oestradiol is not thought to activate the coagulation cascade like oral oestrogen does. Oral oestradiol is one of the most common forms of oestrogen used in HRT. It is converted to estrone in the intestine and liver, which is a potent stimulant of hepatic protein synthesis and in turn, the production of coagulation factors [48]. Oral oestrogen activates thrombin generation through its hepatic first-pass effect [47, 48], faster and to higher levels than the transdermal route [48]. Thrombin is a central component of the coagulation cascade, responsible for converting fibrinogen to fibrin and has been shown to be a marker of thrombotic risk [48]. Furthermore, oral oestrogen is thought to have a greater resistance to activated protein C, thus overall, increasing their thrombotic risk [47]. Other thrombophilic markers are not

thought to be affected by HRT usuage [48].

A few thrombotic occurrences have been reported with progestogen-only hormonal contraceptives. However, progestogen-only therapy is generally presumed to have less risk compared to those containing a combination of oestrogen and progestogen [43]. Progestogens with less androgenic activity are thought to have a positive impact on the coagulation profile [22]. For example, trimegestone has been shown to have a greater fibrinolytic response than dydrogesterone in a randomised multicentre study of 186 women [22]. Similarly, the oestrogen-alone regimes have been shown to have less VTE risk than the combined forms [43].

Micronized progesterone has been shown to have a neutral effect on the vasculature and therefore, not to increase the VTE risk compared with non-users [47].

The mechanism by which oestrogen and progesterone solely or in combination contribute to the increased risk of developing a thrombosis is complex and not fully understood. All oestrogens, regardless of mode of administration, have been found to increase the levels of procoagulant factors such as VII, X, XII, and XIII, and to decrease anticoagulant factors, such as protein S and antithrombin. This leads to a more procoagulant state as it is not balanced by the degree of fibrinolytic activity [43].

We aim to conduct a randomised controlled trial to compare the effects of micronized progesterone and Medroxyprogesterone Acetate on the cardiovascular system, lipid profile and coagulation cascade in women with premature ovarian failure receiving hormone replacement therapy.

Methodology

Trial Duration
Each subject was expected to participate in the trial for a total of 14 months from consent to the final visit allowing for the washout period. The end of the trial will be defined as the last visit of the last patient recruited.

A further 12 months will be required to complete follow-up and analysis of the data.

Definition of Trial Time Measurements
Primary Endpoint

The mean changes in the vascular elasticity.

Secondary Endpoints

1. Mean changes in the pulse waveform patterns
2. Mean changes in the lipid profiles
3. Changes in the coagulation factors
4. Effects on the quality of life of these women.

Table 5: Schedule of events

Visit (months±weeks)	-1±1 Screening	0 ±2 Baseline	3±2 V3	6±2 V4	12±2 V5

Consent	×				
Quality of life questionnaire		×	×	×	×
History (including PMHx and FHx)	×	×	×	×	×
Physical examination		×			
Vital signs (BP/BMI)		×	×	×	×
Pulse waveform		×	×	×	×
Routine safety bloods		×	×	×	
Research blood samples		×	×	×	×
Randomisation		×			
Adverse Events		×	×	×	×
Concomitant Medication		×	×	×	×

Trial Medication
Evorel 50, patches, releasing 50 mcg/24 hours produced by Janssen-Cilag. These patches will be taken by all patients and they will be prescribed at a dose in line with those recommended in the SmPC. For the purposes of the trial, Evorel is not considered to be an IMP.

Utrogestan, oral, 200 mg/24 hours from days 15-26 of a 28 days cycle produced by Ferring.

Medroxyprogesterone Acetate, oral, 10 mg/24 hours from days 16-26/cycle produced by Pfizer.

All drugs were supplied by the local site pharmacy from normal clinic stock in standard packaging. The IMP will be dispensed as part of routine clinical care, therefore, no specific labels will be provided.

Dosing Regimen
Group A was be prescribed: 50 mcg/day patch of oestradiol (Evorel® patch) twice a week in conjunction with Utrogestan® 200 mg/day from days 15-26 of a 28 days cycle, taken orally.
Group B was be prescribed: 50 mcg/day patch of oestradiol (Evorel® patch) twice a week in conjunction with Medroxyprogesterone Acetate 10 mg/day from days 16-26/cycle, taken orally.

The oestradiol levels of all participants were assessed at three months and women with levels less than 200 pgm/l, had their Evorel patch dose increased to 100 mcg/day patches twice a week.

Prior to randomisation, the participants were asked to stop their current treatment regimen for a total of 1 month, to ensure an adequate washout time period has been accounted for. This washout time frame was based on other published trials. It accounts for the half-life of the medication. It was necessary to ensure that previous treatments did not influence or impact on the trial results. There was no evidence to suggest any risk from stopping their current treatment regime for this short duration.
The participants were be involved in the study for a maximum of 14 months from consent to the final visit, but the trial duration will be 12 months.

Number of patients (planned and analysed)

Planned

It was planned to recruit 90 subjects.

Analysed

71 subjects were screened.
68 subjects were randomized.
56 subjects failed to complete all trial related procedures.

Group A: micronised progesterone
Group B: medroxyprogesterone acetate

The numbers for each end point vary:

Pulse Wave Velocity:
Visit 1: 71 x screened
Visit 2: 57 – 29 randomised to Group A; 28 randomised to Group B
Visit 3: 44 – 22 randomised to Group A; 22 randomised to Group B
Visit 4: 36 – 17 randomised to Group A; 19 randomised to Group B
Visit 5: 33 – 18 randomised to Group A; 15 randomised to Group B

Side effects and satisfaction questionnaire:
Visit 1: 71 x screened
Visit 2: 67 – 33 randomised to Group A; 34 randomised to Group B
Visit 3: 50 – 23 randomised to Group A; 27 randomised to Group B
Visit 4: 38 – 18 randomised to Group A; 20 randomised to Group B
Visit 5: 35 – 19 randomised to Group A; 16 randomised to Group B

Coagulation and lipid profile:
Visit 1: 71 x screened
Visit 2: 61 – 32 randomised to Group A; 29 randomised to Group B
Visit 3: 43 – 20 randomised to Group A; 23 randomised to Group B
Visit 4: 37 – 19 randomised to Group A; 18 randomised to Group B
Visit 5: 32 – 17 randomised to Group A; 15 randomised to Group B

Thrombin generation:
44

The reasons for patient withdrawal from the study

Removed due to non-compliance n=2 – 1 from Group A; 1 from Group B
Removed as their symptoms were not controlled n=5 – 4 from Group A; 1 from Group B
Removed as site location was not convenient n=1 – 1 from Group A
Wanted to pursue treatment with egg donation n=1 – 1 from Group B

Main criteria for inclusion

Inclusion Criteria

1. Females aged between 18 and up to 45 years.

2. Confirmed diagnosis of POF with a history of oligomenorrhoea/amenorrhoea for at least 4 months and elevated gonadotrophins ≥ 30mIU/ml on two separate occasions.
3. Willingness to participate.
4. No concomitant co-morbidities that would contraindicate the use of hormonal preparations such as unexplained bleeding, breast cancer, endometrial cancer or thromboembolic disease.

Exclusion Criteria

1. Age under 18 or over 45 years.
2. Pregnant or lactating females.
3. Contraindication to the use of hormonal preparations, such as a history of cerebrovascular disease and thromboembolic disease.
4. Factors present in the past medical history that would contribute to the increased risk of cardiovascular disease, such as, previous myocardial infarction, angina, diabetes, kidney disease, hyperlipidaemia or hypertension.
5. Personal history of thromboembolic episode or known thrombophilia that would impact on the results of the thrombogenic profile.
6. Presence of any other clinically significant medical condition, as determined by the investigators.
7. Known Porphyria.
8. Known liver disease.
9. Known past or suspected breast cancer.
10. Undiagnosed vaginal bleeding.
11. Genital tract carcinoma.
12. Thrombophlebitis.
13. Smokers.
14. Body mass index >35.
15. Known hypertensive disease.
16. Women on concomitant medications that could influence the results, such as anti-hypertensives or anticoagulants.
17. Known intolerance, allergy or contraindication to the use of oestrogen and/or progesterone.
18. Known hypersensitivity to any of the active substances or excipients contained within the utrogestan, Medroxyprogesterone Acetate or Evorel patches
19. Known allergy to peanuts or soya.

Selection of Participants

Participants will be recruited from the Gynaecology Endocrine (Premature Ovarian Failure) Clinic at King's College Hospital NHS Foundation Trust by Dr Monica Mittal and Mr Haitham Hamoda. Only subjects fulfilling the inclusion and exclusion criteria will be included.

Randomisation

The women will be randomly allocated to a treatment group using web-based computer randomisation software, Graph Pad.

Results

Pulse wave velocity and pulse wave analysis:

No statistical difference was seen between the two treatment arms.

Lipid profile:

The percentage change within the lipoprotein profile parameters, throughout the study duration, is minimal but does reach statistical significance for some time periods. Overall, for both micronized progesterone and MPA, after 3-months utilisation, the total cholesterol, LDL, triglyceride and HDL levels are all demonstrated to be lower than the baseline levels, whereas the cholesterol ratio is shown to increase. This trend is maintained after 6-months utilisation, albeit for the triglyceride levels which are shown to increase nominally with MPA usage. After 12-months from the commencement of micronised progesterone, the total cholesterol, LDL, triglyceride levels and cholesterol ratio are all shown to increase from the baseline with lower HDL levels. However, changes in the lipoprotein parameters with MPA, maintain the initial pattern of a lower total cholesterol, LDL, triglyceride and HDL levels from baseline, but a higher cholesterol ratio. The small changes in the lipoprotein parameters from baseline and the lack of statistical significance, limit the ability to draw firm conclusions regarding the impact of micronized progesterone and MPA on CVD risk through an analysis of the lipoprotein parameters in women with POI.

Coagulation cascade and thrombin generation:

This study demonstrated no significant adverse effect or difference on thrombin generation between the two groups. Utrogestan was noted to result in a statistically significant reduction in Protein C, Protein S and Antithrombin III levels with time, with MPA demonstrating a similar trend. The confidence intervals for these differences, however, were wide and the clinical relevance of these small differences requires further evaluation in larger studies. The changes in thrombin generation parameters, however, did not achieve statistical significance for the two subgroups over time.

Side effect profile and women's satisfaction with their HRT regimen:

A common, overarching theme emerges from this survey, that women with POI do perceive an overall improvement in their vasomotor and neurological symptoms such as mood with HRT use incorporating either progesterone component, as has been demonstrated in older naturally menopausal women. However, urogenital symptoms, sexual function and energy levels are not shown to improve in either treatment arm, opening the possibility of a role for testosterone replacement in the management of these symptoms. Furthermore, despite similar adverse effects being expressed by both treatment groups, Utrogestan is demonstrated to be better tolerated. Larger studies incorporating the standardised quality of life questionnaires in this sub select population are required, to allow a direct comparison of the analysis with existing studies that have been conducted in older menopausal women, reducing the degree of heterogeneity.

Statistical Methods

Sample Size

The sample size was calculated using the Altman nomogram [45] and based on changes in the

augmentation index in response to exposure to the two different progesterones assessed in the study. Baseline data were obtained from reference population Pulse Wave Analysis (PWA) and Augmentation Index (AI) data as reported by McEniery et al. [46] based on the reported findings, we considered a change of 8% with Standard Deviation of (12) to be a clinically significant difference to detect. Using the Altman nomogram, this would give a standardised difference of 0.80. A sample of 80 women in two groups would detect a standardised difference of 0.80 with 80% power at the 5% level of significance. We aim to recruit 90 women in total to allow for a 10% drop out and loss to follow up.

Randomisation

Women will be randomised to either treatment arm using web-based computer generated software, Graph Pad.

Analysis

Statistical analysis will be by intention to treat. Demographic and outcome data will be reported separately. Randomisation will be as per the inclusion and exclusion criteria. Variables that are normally distributed will be presented as means and standard deviations and analysed using the independent and paired t test while data not normally distributed will be presented as medians and range and analysed using the Mann Whitney U test. The Chi square or Fisher's exact will be used as appropriate for independent nominal data. Confidence intervals will be used where appropriate and statistical significance will be defined as a p value of less than 0.05.

Every attempt will be made to gather data on all subjects randomised, irrespective of compliance with the treatment protocol. Any deviations from the original plan will be documented and justified in the final report. The flow of patients through the trial will be reported using the CONSORT diagram. The details below are examples only. Complete this section as described in the protocol.

Summary – Conclusions

Demographic data

The trial was conducted in all female subjects aged between 18 and 45.

Safety data

As this was a Type A trial of well-known IMPs given as per standard of care, no AEs were recorded for the trial only symptoms were recorded on a questionnaire at each visit. 3 SAEs, were identified as treatment-emergent and included in the safety analysis. Summary tables for symptoms and SAEs are presented in the appendix of this synopsis.

The proportion of patients that experienced at least one SAE was 4.4% (n=3).

There were no Serious Adverse Reactions (SARs), no unexpected SARs and no SUSARs.

Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 02/Jul/2019.

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APPENDICES

i) Summary of treatment-emergent symptoms in the per protocol population

System Organ Class	Preferred Term	Total Number of Occurrences of a Symptom in the Medroxyprogesterone Arm	Total Number of Occurrences of a Symptom in the Utrogestan Arm
Cardiac disorders	Palpitations	0	1
Gastrointestinal disorders	Nausea	10	9
	Bloating	17	22
	Abdominal Spasms	1	0
	Weight change	13	24
General disorders and administration site conditions	Breast tenderness	12	19
	Itchy	2	0
	Fatigue	3	1
	Sleep deprived	1	0
	Cramps	1	0
	Rash at patch site	0	4
	Itchy eyes	0	1
	Dry mouth	0	1
	Ache in legs	0	1
Infections and infestations	Shingles	1	0
Injury, poisoning and procedural complications	Overdose	0	1

Metabolism and nutritional disorders	Dehydration	0	1
Musculoskeletal and connective tissue disorders	Joint stiffness Back pain	2 1	0 0
Nervous system disorders	Dizziness Headache Low concentration Insomnia Drowsiness	1 20 2 0 0	3 15 0 1 1
Psychiatric disorders	Pre-menstrual symptoms Irritable Mood swings Panic attacks Nightmares	18 25 28 1 0	20 18 17 0 1
Reproductive system and breast disorders	Irregular vaginal bleeding	9	15
Skin and subcutaneous tissue disorders	Acne	6	0

The main concerns (matrix scale score 1-3) of the respondents over the course of the study, categorised by the treatment arm

Symptoms categorised by oestradiol patches in combination with micronised progesterone

Symptoms	Baseline		3 months		6 months		12 months		p-value
	%	n=33	%	n=23	%	n=18	%	n=19	
Low energy levels	39.39	13	47.83	11	55.56	10	47.37	9	0.73
Hot flushes	42.42	14	21.74	5	22.22	4	15.79	3	0.14
Low libido	33.33	11	26.09	6	27.78	5	31.58	6	0.94
Vaginal dryness	24.24	8	21.74	5	16.67	3	26.32	5	0.90
Night sweats	24.24	8	13.04	3	27.78	5	0	0	-
Low mood	3.03	1	0	0	0	0	0	0	-
Asymptomatic	6.06	2	13.04	3	16.67	3	10.53	2	0.67
Hair changes	3.03	1	4.35	1	5.56	1	0	0	-
Sleeping difficulties	0	0	0	0	0	0	0	0	-

Symptoms categorised by oestradiol patches in combination with MPA

Symptoms	Baseline		3 months		6 months		12 months		p-value
	%	n=34	%	n=27	%	n=20	%	n=16	
Low energy levels	50	17	59.26	16	55	11	50	8	0.89
Hot flushes	41.18	14	14.81	4	10	2	12.5	2	0.02
Low libido	20.59	7	29.63	8	35	7	25	4	0.68
Vaginal dryness	20.59	7	0	0	5	1	12.5	2	-
Night sweats	14.71	5	7.41	2	15	3	12.5	2	0.82
Low mood	17.65	6	7.41	2	5	1	6.25	1	0.37
Asymptomatic	5.88	2	11.11	3	5	1	6.25	1	0.83
Hair changes	2.94	1	0	0	0	0	0	0	-
Sleeping difficulties	0	0	0	0	10	2	18.75	3	-

ii) Summary of treatment-emergent SAEs in the study population

System Organ Classification	Serious Adverse Event Details	Number of Subjects Experiencing the SAE in the Medroxyprogesterone Acetate Arm	Number of Subjects Experiencing the AE in the Utrogestan Arm	Related to IMP?	Subject withdrawn from Trial?
Infections and infestations	Shingles	1	0	Not related to IMP	No
Injury, poisoning and procedural complications	Overdose	0	1	Not related to IMP	No
Metabolism and nutrition disorders	Dehydration	0	1	Not related to IMP	No