Metabolic and Neuro-Endocrine Effect of Treating PCOS in Adolescents

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RESEARCH PLAN

A) SPECIFIC AIMS

Treatment recommendations for polycystic ovary syndrome (PCOS) in adolescent girls are based on very few studies and mainly expert opinion.[1] Therefore more studies are needed on therapeutic regimens that improve the wellbeing of adolescent girls suffering from this condition. Targeted intervention that addresses the core metabolic disturbance in PCOS may also provide much needed information about the interconnectivity between signs, symptoms, and diagnostic parameters. The current proposal will provide preliminary data on a novel treatment paradigm for adolescent PCOS as well as important pilot information on neuro-endocrine connection in PCOS.

Hypothesis: A 6-month trial of synergistic combination drug therapy with low dose insulin sensitizer (metformin) and anti-androgen (spironolactone) will significantly improve metabolic profile and clinical manifestations of PCOS in adolescents.

Specific Aim 1: To examine the effects of a 6-month drug intervention with combined low dose metformin (500mg orally BID) and spironolactone (50 mg orally QD) in adolescent PCOS on biochemical markers of insulin resistance such as the whole-body insulin sensitivity index (WBISI). The WBISI will be calculated from glucose and insulin values obtained during the oral glucose tolerance testing (OGTT) before and after drug intervention.

While improving insulin resistance is an important metabolic target, most patients are concerned with central adiposity, which is linked to insulin resistance. To this end, studies have demonstrated reduction in body mass index (BMI), and an improvement in waist-hip circumference in women treated with metformin [2]. Therefore, secondary treatment outcomes will not only include lipid profile and other biochemical markers of PCOS but also include changes in body composition as assessed by bio-impedance scale. Further clinical outcome measures will include menstrual cycle regularity, ovarian morphology changes as assessed by trans-abdominal ultrasound, hirsutism scores and resting blood pressure.

Specific Aim 2: To examine structural and functional brain changes associated with adolescent PCOS before and after drug intervention designed to improve insulin resistance.

In adolescents, improving insulin resistance with metformin was recently attributed to reduced food intake with decreased perceived hunger [3]. Therefore, our second aim may lead to a mechanistic understanding of why adolescent with PCOS often improve BMI with improved insulin resistance. Indeed, it has been postulated that insulin resistance not only affects diabetes and cardiovascular disease but exerts a significant impact on central nervous system (CNS) function regarding neuro-functional responses to food-related cues. Insulin resistant women with PCOS show a differential brain response to glucose challenge testing. These authors hypothesize this could lead to increased caloric intake and eating past the point of satiety (non-homeostatic eating) [4]. Since food intake is also a function of mood, a standard depression questionnaire will be administered.

B) BACKGROUND AND SIGNIFICANCE

PCOS is the most common and complex endocrinopathies in the female population. At the core of the condition lies an exaggerated ovarian/adrenal androgen production in response to physiologic and supra-physiologic elevations in insulin. Clinically, this hyperandrogenism is linked to acne, hirsutism, and menstrual irregularity. Additionally, hyperandrogenism positively feeds back onto insulin resistance, thereby perpetuating hyperinsulinemia. This becomes a vicious cycle, and it is this perpetuated insulin resistance that is deemed responsible for the detrimental metabolic sequelae of PCOS. Adverse outcomes include Type 2 diabetes, cardiovascular disease, depression, and obstructive sleep apnea (OSA). Adolescent PCOS treatment focuses on treating insulin resistance (i.e., metformin) with or without symptomatic/cosmetic relief through oral contraceptive hormones. However, studies seeking significant metabolic improvement by targeting both hyperandrogenism and insulin resistance are lacking.

Without treating hyperandrogenism in PCOS patients, insulin resistance remains. This is because the driving force behind the metabolic downward spiral is not shut down. Therefore, simultaneously targeting *both* insulin resistance and hyperandrogenism should be highly effective in improving biochemical markers of insulin resistance in adolescents with

PCOS. Indeed, reports in adolescents [5] indicate that hyperandrogenism is an important treatment target in the goal of improving metabolic health parameters. A recent study in adult women with PCOS demonstrated that the drug combination of low dose anti-androgen (spironolactone) and low dose insulin sensitizer (metformin) is superior to either drug alone in improving all metabolic parameters of PCOS [6]. Remarkably, the metformin dose and spironolactone dose used in this study was lower than the usual recommended dose for mono-therapy in adolescents with PCOS.

Spironolactone has been used for decades as a potassium sparing diuretic and was found to have anti-androgenic effect in the 1970's [7]. Its anti-androgenic effect is carried out through a dual mechanism of androgen receptor blockade, and via spironolactone's inhibition of 5-alpha reductase, thus reducing the conversion of testosterone to its biologically more potent metabolite, di-hydrotestosterone. The concern with studying spironolactone in adolescent girls has always been the potential effect of the drug to under-virilize a male embryo through transplacental exposure of the drug, should an unplanned pregnancy occur. However, unlike the widely prescribed OC, spironolactone does not pose a serious health risk (i.e thrombo-embolic events) to the patient herself [8] and therefore is the safer drug for the subject, especially if she is overweight and has a family history of thrombo-embolism events. Furthermore, a recent study in adolescent girls with hirsutism, demonstrated that a carefully designed study does not need to preclude adolescents from receiving anti-androgen therapy [9]. In this study, adolescent girls with hirsutism were treated with the potent anti-androgen finasteride for 6 months and were not on oral contraceptive pills. In our proposed study, risk will be reduced by precluding sexually active teens from partaking in the study. Furthermore, all participants, even if not sexually active will be required to take a monthly pregnancy test for the duration of the study. This would likely safeguard against any inadvertent exposure of a male conceptus to spironolactone, as study drug would be discontinued immediately in the event of a positive pregnancy test. Lastly there has been a case report on a young woman treated with spironolactone (200-400mg daily) throughout each of her pregnancies due to Bartter Syndrome. In the case description she delivered two normal male infants and one normal female[10]. At the time of the report one of the males had entered puberty at a physiologic time. While the teratogenic concern remains based on some, but not all animal studies[11,12] carefully selected subjects and stringent protocol adherence should allow this very beneficial drug to be examined in adolescents with PCOS.

Metformin is well known for its insulin sensitizing effect at the level of the liver and its beneficial effect on diabetes prevention [13]. In a double- blind placebo-controlled study in obese youth the principal investigator of the current proposal has shown that metformin is well tolerated in youth and has beneficial effects on BMI and autonomic control of the heart [14]. Metformin use, especially in adolescents, is becoming an increasingly preferred treatment over oral contractive pills unless the patient is sexually active. In their 2013 guidelines on treating PCOS in adolescents, the Endocrine Society states that metformin has shown to restore menstrual regularity, improve hyperandrogenism and glucose tolerance in obese and non-obese patients [1]. However, due to a lack of sufficient adolescent studies, the Endocrine Society's first line treatment recommendation remains oral contraceptive pills for PCOS in adolescents. The safety profile of metformin is excellent and the risk for lactic acidosis in patients with normal kidney function is no longer a concern for the medical community. Gastrointestinal side effects are not uncommon but can be overcome by taking the metformin with food and by starting with a low dose that is incrementally increased, titrated to tolerance.

C) PRELIMINARY STUDIES/EVIDENCE OF CAPABILITY

The principal investigator has a longstanding history in clinical research in the field of adolescent obesity as it relates to metabolic and cardiovascular disease. She has specifically performed drug interventions with metformin [14] and studied adolescent girls with PCOS [15], giving her great expertise in proposed area of study. She has a track-record of publishing as first and senior author and has obtained NIH funding (K23).

At her prior employment she established and directed a successful multi-specialty adolescent PCOS program which allowed her to recruit 100 subjects for her study on oral glucose tolerance in adolescents with PCOS. Since her arrival at CMH last year, she has recreated a similar program at CMH's Broadway location. For the program Dr. Burgert has established collaborations with adolescent medicine, radiology and nutrition services. All patients seen in her program have OGTT and ovarian ultrasound performed at the same day of their initial consultation. Patients and families enjoy the "one stop" complete evaluation. This model not only allows for streamline care but also makes it easier to offer studies that require these examinations.

Dr. Burgert is becoming a nationally recognized expert in PCOS and was just selected to give the "Year in Review in PCOS" at the upcoming annual PES/PAS meeting. She has written chapters and reviews on the topic and is the education chair for the AES-PCOS society. She recently returned from presenting her data on ovarian ultrasound in adolescent PCOS at the AES-PCOS society in Italy.

Dr. Bruce is a licensed clinical psychologist with advanced training in cognitive neuroscience and functional neuro-imaging. Her laboratory investigates how the human brain weighs risks and benefits of choices about food (i.e. pediatric eating behaviors), purchasing decisions (i.e., neuroeconomics), and adherence to medical regimens (i.e., pharmacologic interventions in neurologic populations).

Her research has revealed that obese youth demonstrate differential brain structural and functional profiles when compared to healthy weight youth. Specifically, in response to appetitive stimuli including food images, obese children exhibiting increased reactivity to food cues in limbic and paralimbic cortical regions[16]. Equally disconcerting, these differences also extend to food advertising cues (i.e., fast food brand logos) with obese children having a more limbic response and lean children demonstrating more reactivity in the prefrontal cortex (PFC), a cortical region associated with self-control [17]. Even in the resting state (without stimulatory food cues) the brain's neurofunctional connectivity of obese children differs from that of lean children, with obese children demonstrate increased connectivity between cortical regions associated with reward and valuation. In regards of applying Dr. Bruce's findings to PCOS, nothing is known about neurofunctional response to external appetitive cues in PCOS. Even less is known as to how pharmacologic treatment (i.e. metformin and spironolactone), can directly or indirectly impact brain structure and function.

RESEARCH DESIGN AND METHODS

Research Design and Procedures:

The overall objective of this proposal is to determine if metabolic improvements occurs in adolescents with PCOS after a 6-month open label intervention with a low dose synergistic drug regimen of metformin (insulin sensitizer) and spironolactone (anti-androgen).

Initial data will be collected as part of the patient's routine PCOS health care. Should a patient consent to the study this data will be used as baseline analysis. During the 6- month intervention study the subject will have a safety monitoring visit at 3 months into the intervention which will include blood work, complete examination and compliance history. All baseline examinations will be covered by insurance. All post intervention biochemical or other testing will be covered by study funds and Dr. Bruce's neuro-imaging start- up funds.

For complete study design: see flow chart at end of the RESEARCH DESIGN AND METHODS section.

Study Design:

This is a single center clinical research study to obtain pilot data.
It is a non-controlled open label drug intervention study in a convenient sample of adolescent girls attending a PCOS clinic.

Patients/Subjects:

The sample will include 20 adolescent females (ages 13-21) of all ethnic backgrounds referred to the CMH adolescent PCOS program for evaluation and management of PCOS.
Patient risks and safeguards are extensively described in PROTECTION OF HUMAN SUBJECTS

Inclusion Criteria: Generally healthy; Meeting Androgen Excess Society (AES) diagnostic criteria of PCOS: Menstrual Dysfunction or PCO ovaries on ultrasound AND clinical or biochemical hyperandrogenism.
Normal liver and kidney function. No chronic illnesses except for stable, treated hypothyroidism.

Exclusion Criteria: Use of metformin and/or spironolactone within the last 6 months. Currently on either oral hormonal contraception or other forms of hormonal contraception such as Depo-Provera^{R}, NuvaRing^{R}

Current or past pregnancy; Currently sexually active; Psychiatric disorder based on self/parental report; type 2 diabetes (blood glucose > 200mg/dl on OGTT); Anemia (Hct < 35); Baseline creatinine > 1.0 mg; Abnormal liver transaminases > 2 x the upper limit of normal range, potassium elevated outside the reference range (in non-hemolyzed blood sample).

Drugs/Experimental Intervention:
Subjects with receive 6 months combination drug therapy with a combination of low dose metformin and spironolactone.

Intervention – Metformin: The investigational pharmacy will distribute the metformin. The study dose will be 500 mg/tab orally BID, to be taken with food at breakfast and dinner. In order to improve tolerability of the regimen, the drug will be started sub-therapeutically. The first 4 days only ½ tablet will be taken with dinner and then increased to 1 tab for the next 3 days with dinner. The second week ½ tablet will be added to breakfast for four days and then the next 3 days the breakfast dose is increased to 1 tablet. After two weeks the subject will be on 500 mg BID from which time the 6- month intervention is counted. Long term use of metformin may lead to vitamin B12 deficiency. Therefore, vitamin B12 levels will be measured in the study and balanced diet is recommended.

Intervention - Spironolactone: The investigational pharmacy with distribute the spironolactone. The subject will take one tablet (50mg/tab orally QD) with breakfast once the metformin dose has been titrated up to 500 mg BID, approximately 2 weeks into the study. The 6-month intervention counts from the first dose of spironolactone taken.

Observation and Measurements:

Whole Body Insulin Sensitivity Index (WBISI): This determinant of insulin sensitivity has been validated against the euglycemic clamp as an accurate measure of insulin sensitivity in adolescents by the PI's former research group.[18].The WBISI is calculated based on the area under the curve (AUC) for glucose and insulin during an oral glucose tolerance test. While the original WBISI (also known as Matsuda Index)[19] requires a 3 hour glucose tolerance, calculating the index during a 2 hour glucose tolerance test has become common practice with good results [20].
Procedure: After an overnight fast, an 18-22 gauge intravenous catheter will be placed to allow easy access and minimize discomfort during repeated blood sampling. All hormone labs and baseline metabolic labs (including the 0- minute glucose and insulin) will be drawn immediately after placement of the indwelling catheter. The subject will be asked to drink 8 oz of a cola or orange flavored glucose solution (containing 75grams of glucose) to start the OGTT. Blood sampling during the OGTT will occur at the 30-, 60- and 120- minute time points and include blood for glucose and insulin so that the AUC can be calculated for WBISI. The baseline OGTT will be billed to insurance, the post intervention OGTT will be covered by study funds.

Ovarian Ultrasound: This ultrasound will be performed trans-abdominally through a full bladder on the same day of the OGTT in the Endocrine clinic office on Broadway. The radiology tech will use a portable ultrasound for patient convenience. The first ultrasound will be billed to insurance, the post intervention ultrasound will be covered by study funds.

Bio-impedance Scale: Body composition will be measured through the bio-impedance scale available in the Endocrine Clinic.

Depression Scale: The Hospital Anxiety and Depression (HADS) assessment will be used. The instrument has adequate psychometric properties and is considered a valid and reliable tool for screening adolescents for depression. A member of the MAPP research/clinical team has successfully implemented this tool in her examination of overweight insulin-resistant adolescents [21].

Data Analysis:

Primary Outcome: Insulin Sensitivity as assessed by the whole- body insulin sensitivity index (WBISI) also known as the Madsuda index. This is calculated from the Oral Glucose tolerance test and is a better parameter of insulin sensitivity than HOMA (based on fasting glucose and insulin values).

Power Analysis: Based on published research studies, a sample size of N = 20 patients per group, shows sufficient power. Metformin + Spironolactone group (n = 62): WBISI improved from 4.7+- 2.71 to 8.04 +- 4.03.
When the sample size is 20, a paired *t*-test with a 0.05 two-sided significance level will have 93% power to detect the difference between a before treatment WBISI of 4.700 and after-treatment WBISI mean of 8.000, assuming that the standard deviation of change in WBISI is 4.0.

PROTECTION OF HUMAN SUBJECTS

Subject Population Recruitment

The study population will include adolescent females (age 13 to 21 years old) referred to Children's Mercy Adolescent PCOS Program. Prior to her first visit, the patient and her family will receive a letter about the voluntary research studies available through the PCOS Program. The patient may decline hearing about our research during her initial visit or any visits to the PCOS Program. If she declines participation, she will continue to receive standard of care in the Adolescent PCOS Program.

Medical Information Technology (MIT) will provide a list of qualified candidates. Potential subjects will be identified after a review of medical records through Cerner or other CMH sources (databases, billing records, pathology reports, admission logs). This may involve access to records by individuals not involved in the patient's care.

All subjects diagnosed with PCOS and treated at the CMH Diabetes Clinic will be identified by ICD-9 diagnostic codes. In accordance with 45 CFR 164.512(i)(1)(ii), (see http://www.hhs.gov/hipaafaq/permitted/research/317.html), qualifying patients will be identified initially by creation of a PCOS pre-screening log. This information is to be used solely for work preparatory to research (in this case the identification of qualifying subjects for targeted screening). No pre-screening information will be removed from CMH. This is necessary in order for the investigator, sub-investigator(s) and research coordinator(s) to effectively approach the entire clinic population for recruitment into the study. With the assistance of the IT department, all current CMH patients with type 1 diabetes [defined by (1) any patient visit tagged the diagnostic codes for PCOS from the prior 24 months will be identified. A password-protected pre-screening log of qualifying subjects and their next scheduled clinic appointment to the CMH diabetes center will be maintained to aid in subject identification. This will be updated quarterly. This will aid the key study personnel in efficiently and effectively attending particular clinic days/locations for optimal recruitment. The type 1 diabetes pre-screening log will contain subject names, medical record numbers and future clinic dates, as well as coordinator/investigator notes regarding eligibility. The entire screening log will be destroyed upon completion of targeted enrollment.

Qualified patients may also be identified by review of the daily clinic schedule, via review of Cerner data and billing records provided by MIT. Whether by phone or in person, only study personnel will give details about the study to subjects and families.

At their clinic visit, the study will be explained briefly, and subjects/parents will be asked whether or not they are interested in participating. All interested subjects and parents will have the study (including the risks and benefits) thoroughly explained using the written consent. They will be given copies of the consent to review without staff present, in order to allow them time to discuss with each other. They will be given an opportunity to ask additional questions after they have read the consent. If subject and parent agree to participate the appropriate child or adult consent will be signed.

Alternatively, subjects and parents will be given the opportunity to take consents home for further reading, discussion, and decision-making at a later date. These families may enroll at their next scheduled visit or another time convenient for them. No pressure will be placed on any subject or family to enroll. These families may be contacted by phone to ask if they want to participate.

Refusal to participate will be notated in the Research Prescreening Log. These patients will not be approached additional times for participation in this study.

A password-protected pre-screening log of qualifying subjects and their next scheduled clinic appointment to the CMH diabetes center will be maintained to aid in subject identification. This will aid the study personnel in efficiently and

effectively attending particular clinic days/locations for optimal recruitment. The PCOS pre-screening log will contain subject names, medical record number, date of birth, clinic visit date, clinic location, clinic provider appointment time as well as coordinator/investigator notes regarding eligibility. For those individuals not interested in participating, the minimum amount of PHI will be retained to ensure they are not approached again regarding participation in this study. Only their name and MRN will be retained. The remainder of the information will be deleted from the file. The entire screening log will be destroyed upon completion of targeted enrollment.

INFORMED CONSENT

If the subject is between the ages of 13-21 on the date of the clinic appointment/study visit and parent agree to participate in the study, written informed consent/assent will be obtained before the extraction of any CMH EMR data.

Consent/assent will be obtained from research staff at the time of a routine clinical follow-up for T1DM. The study will again be explained to the family/patient and informed consent will be obtained in accordance with CMH Policy, IRB Protocols and Health and Human Services Regulation 45 CFR 46.116.

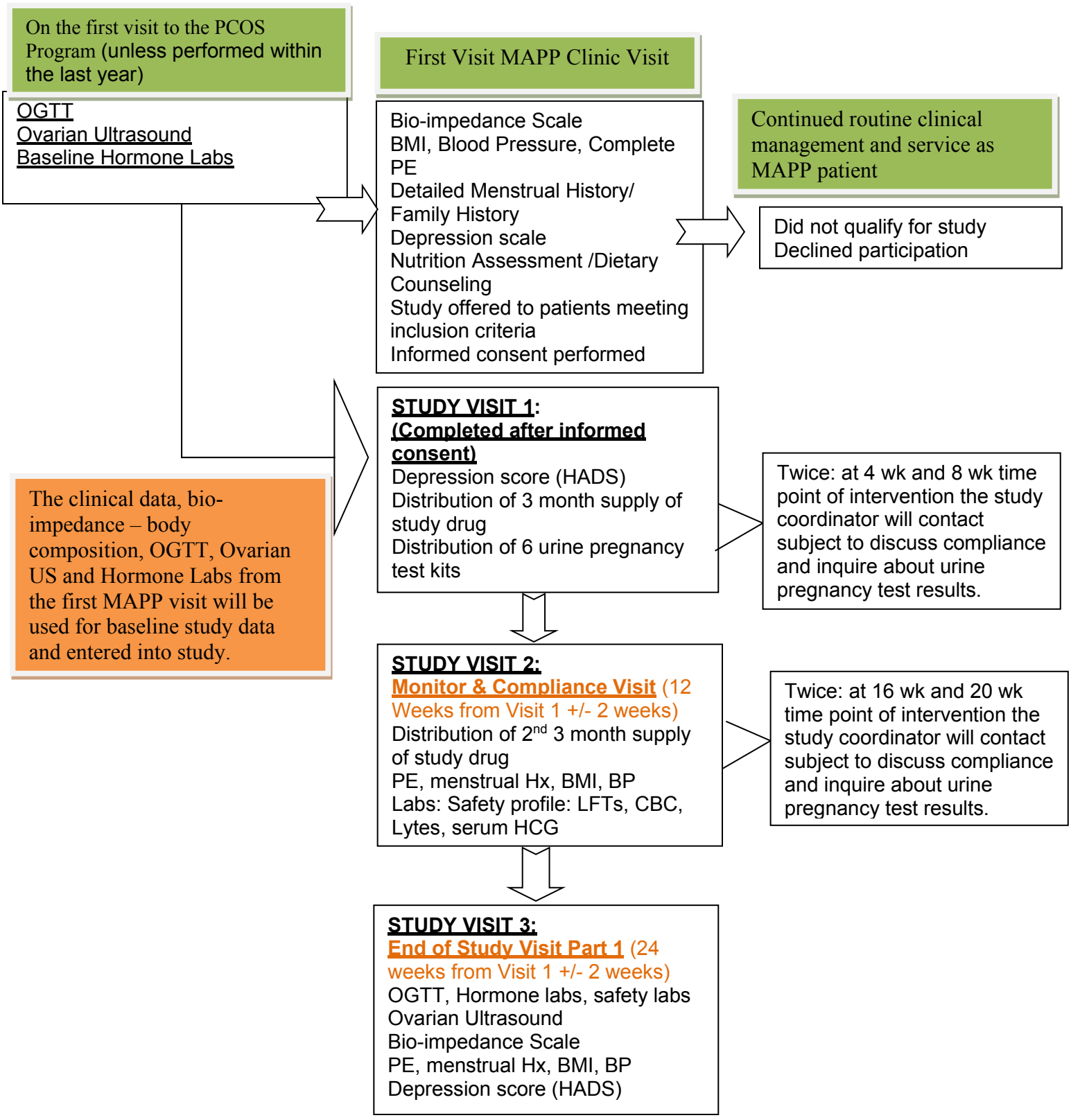
For those patients scheduled to see a provider participating the study, consent/assent and enrollment will be undertaken by a research assistant or alternative provider (and not the medical staff participating in the patient's clinical cares).

If the subject wishes to have more time to consider participating in the study, we will follow-up with a phone call and consent them over the phone at that time if they want to participate in the study.

DATA Collection

Research material will be obtained through from their medical record for laboratory test results including OGTT, anthropometric measures, ultrasound data, vital signs, physical examination, including hirsutism score and birth history, medical history, family history and menstrual history will also be collected. Further materials include a depression score.

VISIT FLOW


On the first visit to the PCOS Program (unless performed within the last year)
OGTT
Ovarian Ultrasound
Baseline Hormone Labs
First Visit MAPP Clinic Visit
Bio-impedance Scale
BMI, Blood Pressure, Complete PE
Detailed Menstrual History/ Family History
Depression scale
Nutrition Assessment /Dietary Counseling
Study offered to patients meeting inclusion criteria
Informed consent performed
Continued routine clinical management and service as MAPP patient
Did not qualify for study
Declined participation
The clinical data, bio-impedance – body composition, OGTT, Ovarian US and Hormone Labs from the first MAPP visit will be used for baseline study data and entered into study.
STUDY VISIT 1: (Completed after informed consent)
Depression score (HADS)
Distribution of 3 month supply of study drug
Distribution of 6 urine pregnancy test kits
Twice: at 4 wk and 8 wk time point of intervention the study coordinator will contact subject to discuss compliance and inquire about urine pregnancy test results.
STUDY VISIT 2:
Monitor & Compliance Visit (12 Weeks from Visit 1 +/- 2 weeks)
Distribution of 2nd 3 month supply of study drug
PE, menstrual Hx, BMI, BP
Labs: Safety profile: LFTs, CBC, Lytes, serum HCG
Twice: at 16 wk and 20 wk time point of intervention the study coordinator will contact subject to discuss compliance and inquire about urine pregnancy test results.
STUDY VISIT 3:
End of Study Visit Part 1 (24 weeks from Visit 1 +/- 2 weeks)
OGTT, Hormone labs, safety labs
Ovarian Ultrasound
Bio-impedance Scale
PE, menstrual Hx, BMI, BP
Depression score (HADS)

Risks and Inconveniences:

1. Phlebotomy/OGTT
While many patients with PCOS would potentially have an OGTT as part of their regular evaluation, most would not have repeat OGTT at 6 months. The OGTT will require venipuncture/i.v. insertion at the commencement of the OGTT. This can be painful may not succeed on first attempt. Bruising and tenderness can occur after venipuncture/i.v. insertion. Some also can feel faint or dizzy during venipuncture. After drinking the standard 75g of glucola on an empty stomach some patients may feel nauseated.

2. Ultrasound
Transabdominal ultrasound evaluation of the ovaries requires a full bladder which may cause subjects discomfort as they are avoiding urination. Furthermore, pressing the ultrasound probe on a full bladder may be uncomfortable for some. There is no radiation or contrast involved in this test.

3. Metformin
The main side effect of metformin is gastrointestinal discomfort such as flatulence, queasiness, and loose bowel movements. Metformin therapy can cause malabsorption of vitamin B12 in the distal ileum after long-term use for several years. Therefore, vitamin B12 levels will be monitored in the study. Subjects will be recommended to stay on a well-balanced diet. Lactic acidosis is a rare but potentially fatal metabolic consequence of metformin treatment. However nearly all reports of lactic acidosis are related to treatment in a patient in which metformin therapy was contraindicated due to renal and liver dysfunction. In the absence of contraindications lactic acidosis is not of concern in otherwise healthy people.

4. Spironolactone
In generally this drug is very well tolerated and poses no health risk in low dose. Given that spironolactone is a potassium-sparing diuretic it may elevate potassium levels in those with underlying kidney disease or excessive potassium intake. In the absence of contraindications like renal failure and in a presence of healthy well-balanced diet, spironolactone is unlikely to increase potassium levels outside the normal range. Should a subject not maintain a normal hydration status she may feel dizzy/lightheaded. This can be avoided by normal fluid intake. The main concern is the potential of this drug to under-virilize a male conceptus. Therefore, sexually active teenagers are excluded from the study. Nevertheless, every subject will be monitored with monthly urine pregnancy tests to assure that the study drug is not continued beyond the first two weeks post conception should an unexpected pregnancy occur. Study drugs will be immediately discontinued should a positive pregnancy test have occurred.

5. Anxiety and Depression Survey risks:

The survey asks sensitive questions about mental health. To reduce discomfort while answering questions, you will answer questions in a private room. Participants are free to not answer any questions. Confidentiality risks are minimal and unlikely to occur. Your child's confidentiality will be protected to the greatest extent possible.

If subject reports any concerning symptoms, we will provide referrals and information on how to get help.

Protection of Human Subjects

All studies will be performed under the direct supervision of either the PI or co-investigators. Phlebotomy discomfort will be minimized by personnel skilled in venipuncture of children and obese patients.

Gastrointestinal side effects with metformin will be minimized by slow incremental dose increases over the course of two weeks. The PI has extensive experience with metformin and has used it in a clinical trial published in 2008. In terms of spironolactone treatment, potential subjects will be interviewed separately from their parents with our Adolescent Medicine co-investigators regarding their sexual activity. A patient who is sexually active will be excluded from participating in the study. Furthermore, subjects will be counseled on the potential teratogenic effects of spironolactone and the requirements of monthly pregnancy tests. Should a subject not comply with monthly pregnancy testing, then she will be discontinued from the study. Should a patient decide to become sexually active during the study, she will also be

discontinued from the study. Should a subject's pregnancy test turn positive then she will be discontinued of both intervention drugs. At that point the principal investigator will meet with the IRB to discuss recommendations in terms of following pregnancy outcome.

Prior to commencing spironolactone/metformin the PI will assess kidney functions and examine electrolytes with attention to potassium levels. Any abnormalities in one or both of these areas will prohibit entry into the trial. Subjects will be given the number of the principal investigator and the RA. They will also be given an emergency number that is available at all times through the section of Pediatric Endocrinology. All on call staff will be aware of the study.

Data and Safety Monitoring Plan

The proposed study poses moderate risk to the subjects. However, it offers the prospect of direct benefits to the subjects. The definition of moderate risk is as follows: Risks are recognized as being greater than minimal, but not high, and there is adequate surveillance and protections to discover adverse events promptly and to keep their effects minimal.

Attribution of Adverse Events

- **Definite:** Adverse event(s) will clearly be related to investigational agent(s) or other intervention.
- **Probable:** Adverse event(s) will likely be related to investigational agent(s).
- **Possible:** Adverse event(s) may be related to investigational agent(s).
- **Unlikely:** Adverse event(s) will doubtfully be related to investigational agent(s).
- **Unrelated:** Adverse event(s) will clearly not be related to the investigational agents(s).

Plan for Grading Adverse Events

0 No adverse event or within normal limits
1 Mild adverse event
2 Moderate adverse event
3 Severe adverse event resulting in hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.
4 Life-threatening or disabling adverse event
5 Fatal adverse event

Plan for Reporting and Reviewing Serious Adverse Events

Serious *unanticipated* adverse events will be reported immediately to the IRB. Serious *anticipated* adverse events where magnitude or frequency exceeds expectations will be reported to the IRB immediately.

The principal investigator will evaluate the adverse event and determine whether the adverse event affects the Risk/Benefit ratio of the study and whether modifications to the protocol (at Risks to Subjects, or in Procedures) or consent form (at Risks and Inconveniences) are required.

Plan for Reviewing and Reporting Non-Serious Unanticipated and Anticipated Adverse Events.

The principal investigator will conduct a review of all adverse events at the half-way point through the study. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

A summary of the adverse events will be reported to the IRB periodically or, at minimum, when re-approval of the protocol is sought. The summary will include number of subjects enrolled and a summary of graded adverse events to date.

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5

	UNA TP	ATP	UNA TP	ANT	UNA TP	ANT	UNA TP	ANT	UNA TP	ANT
Unrelated										
Unlikely										
Possible										
Probable										
Definite										
Totals										
Total Subjects Enrolled to Date										

Data Monitoring and Safety Reviews

The principal investigator (TSB) is responsible for monitoring the data and conducting safety reviews. Either the principal investigator or the IRB have the authority to stop or modify the study.

The principal investigator will conduct a data and safety review at least semi-annually or at the time of re-approval and at any time a serious adverse event occurs. During the review process, the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment.

Confidentiality

The identification of all subjects will be kept confidential and will not be divulged in any publication emanating from this work. Data will be released only upon written consent of the patient.
The study will comply with HIPAA guidelines regarding confidentiality of patient data. All data are labeled with a study ID, including forms and specimens. All data transferred to the database identify the patient only with the study ID. A file on each patient that includes personal identifiers, linking name and contact information to the study ID will be kept. Patient files are kept in secure locations and the clinical center is responsible for taking every other reasonable measure (those set by the state, the site, and the study) to ensure and maintain record confidentiality and patient privacy.

Potential Benefits

The study may have direct benefit to the subjects. Some subjects may experience clinical benefits with the drug intervention such as regularization of menses, reduction in BMI and decrease in acne and hirsutism. Mood may also improve with treatment.

REFERENCES

RESEARCH PLAN:

1. Legro RS, Arslanian SA, Ehrmann DA, et al. Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *The Journal of clinical endocrinology and metabolism.* Dec 2013;98(12):4565-4592.
2. Glintborg D, Altinok ML, Mumm H, Hermann AP, Ravn P, Andersen M. Body composition is improved during 12 months' treatment with metformin alone or combined with oral contraceptives compared with treatment with oral contraceptives in polycystic ovary syndrome. *J Clin Endocrinol Metab.* Jul 2014;99(7):2584-2591.
3. Adeyemo MA, McDuffie JR, Kozlosky M, et al. Effects of metformin on energy intake and satiety in obese children. *Diabetes, obesity & metabolism.* Apr 2015;17(4):363-370.
4. Van Vugt DA, Krzemien A, Alsaadi H, Frank TC, Reid RL. Glucose-induced inhibition of the appetitive brain response to visual food cues in polycystic ovary syndrome patients. *Brain research.* Apr 16 2014;1558:44-56.
5. Alemzadeh R, Kichler J, Calhoun M. Spectrum of metabolic dysfunction in relationship with hyperandrogenemia in obese adolescent girls with polycystic ovary syndrome. *European journal of endocrinology / European Federation of Endocrine Societies.* Jun 2010;162(6):1093-1099.
6. Ganie MA, Khurana ML, Nisar S, et al. Improved efficacy of low-dose spironolactone and metformin combination than either drug alone in the management of women with polycystic ovary syndrome (PCOS): a six-month, open-label randomized study. *J Clin Endocrinol Metab.* Sep 2013;98(9):3599-3607.
7. Stripp B, Taylor AA, Bartter FC, et al. Effect of spironolactone on sex hormones in man. *J Clin Endocrinol Metab.* Oct 1975;41(4):777-781.
8. Shaw JC, White LE. Long-term safety of spironolactone in acne: results of an 8-year followup study. *Journal of cutaneous medicine and surgery.* Nov-Dec 2002;6(6):541-545.
9. Tartagni MV, Alrasheed H, Damiani GR, et al. Intermittent low-dose finasteride administration is effective for treatment of hirsutism in adolescent girls: a pilot study. *Journal of pediatric and adolescent gynecology.* Jun 2014;27(3):161-165.
10. Groves TD, Corenblum B. Spironolactone therapy during human pregnancy. *American journal of obstetrics and gynecology.* May 1995;172(5):1655-1656.
11. Hecker A, Hasan SH, Neumann F. Disturbances in sexual differentiation of rat foetuses following spironolactone treatment. *Acta endocrinologica.* Dec 1980;95(4):540-545.
12. Rose LI, Regestein Q, Reckler JM. Lack of effect of spironolactone on male genital development. *Investigative urology.* Sep 1975;13(2):95-96.
13. Knowler WC, Barrett-Connor E, Fowler SE, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England journal of medicine.* Feb 7 2002;346(6):393-403.
14. Burgert TS, Duran EJ, Goldberg-Gell R, et al. Short-term metabolic and cardiovascular effects of metformin in markedly obese adolescents with normal glucose tolerance. *Pediatric diabetes.* Dec 2008;9(6):567-576.
15. Flannery CA, Rackow B, Cong X, Duran E, Selen DJ, Burgert TS. Polycystic ovary syndrome in adolescence: impaired glucose tolerance occurs across the spectrum of BMI. *Pediatric diabetes.* Feb 2013;14(1):42-49.
16. Bruce AS, Holsen LM, Chambers RJ, et al. Obese children show hyperactivation to food pictures in brain networks linked to motivation, reward and cognitive control. *International journal of obesity.* Oct 2010;34(10):1494-1500.
17. Bruce AS, Lepping RJ, Bruce JM, et al. Brain responses to food logos in obese and healthy weight children. *The Journal of pediatrics.* Apr 2013;162(4):759-764 e752.

18. Yeckel CW, Weiss R, Dziura J, et al. Validation of insulin sensitivity indices from oral glucose tolerance test parameters in obese children and adolescents. *The Journal of clinical endocrinology and metabolism.* Mar 2004;89(3):1096-1101.
19. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes care.* Sep 1999;22(9):1462-1470.
20. DeFronzo RA, Matsuda M. Reduced time points to calculate the composite index. *Diabetes care.* Jul 2010;33(7):e93.
21. Platt AM, Egan AM, Berquist MJ, Dreyer ML, Babar G, Ugrasbul F. Health-related quality of life, depression, and metabolic parameters in overweight insulin-resistant adolescents. *Journal of pediatric health care : official publication of National Association of Pediatric Nurse Associates & Practitioners.* Mar-Apr 2013;27(2):120-126.
22. Ibanez L, De Zegher F. Flutamide-metformin plus an oral contraceptive (OC) for young women with polycystic ovary syndrome: switch from third- to fourth-generation OC reduces body adiposity. *Human reproduction.* Aug 2004;19(8):1725-1727.
23. Ibanez L, Diaz M, Sebastiani G, et al. Treatment of androgen excess in adolescent girls: ethinylestradiol-cyproteroneacetate versus low-dose pioglitazone-flutamide-metformin. *The Journal of clinical endocrinology and metabolism.* Nov 2011;96(11):3361-3366.
24. Ibanez L, de Zegher F. Low-dose flutamide-metformin therapy for hyperinsulinemic hyperandrogenism in non-obese adolescents and women. *Human reproduction update.* May-Jun 2006;12(3):243-252.
25. Burgert TS. Glucose and insulin metabolism in obese youth. *Pediatric endocrinology reviews : PER.* Dec 2006;3 Suppl 4:555-559.
26. Burgert TS, Dziura J, Yeckel C, et al. Microalbuminuria in pediatric obesity: prevalence and relation to other cardiovascular risk factors. *International journal of obesity.* Feb 2006;30(2):273-280.
27. Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *The Journal of clinical endocrinology and metabolism.* Nov 2006;91(11):4287-4294.
28. Bruce AS, Bruce JM, Ness AR, et al. A comparison of functional brain changes associated with surgical versus behavioral weight loss. *Obesity.* Feb 2014;22(2):337-343.

Appendix A

ULTRASOUND ACQUISITION FOR CMH IRB STUDY 16010020

Before the exam:

1. Ensure the participant has a semi-full bladder for appropriate resolution.

Right and Left Ovary

1. Identify ovary in **longitudinal plane**. Optimize focus and depth.
2. Place the label (side, plane) in top left hand corner of the screen so it does not obstruct the ovary.
3. Capture a cine loop through the ovary in the longitudinal plane, starting midline and moving laterally.
4. Identify ovary in **orthogonal (transverse) plane.** Optimize image quality like above.
5. Capture a cine loop through the ovary in the orthogonal (transverse) plane, starting inferior to the ovary moving superior towards the head.
6. Obtain still images of the ovary in the longitudinal and transverse (orthogonal) planes.
    a. Measure and record the length and width of the ovary in each plane.

Uterus

1. Identify the longitudinal plane of the uterus. Optimize focus and depth. Gain can be adjusted, if required.
2. In the midsagittal plane, capture an image of the endometrial lining.
    a. Measure and record the endometrial thickness.
3. Obtain a cineloop through the uterus in the sagittal plane.

Export the Exam: Entire Participant Exam File – may be performed in bulk or ongoing

1. Unlock the USB key with the key code/ password and insert it into the drive on the side of the ultrasound machine. A blue light will flash on the side of the key to acknowledge that the device has been activated.

2. Press the “New Patient Exam” hard key.

3. Click “Archive” from the *Patient Archive* menu on the left side of the screen.

4. Click “Export” from the *Data Transfer* menu on the left side of the screen.

5. The Save As screen will appear.
    a. From the “Save in:” drop-down menu, select the USB key.
    b. Type the Participant ID into the “File Name:” field.
    c. From the “Save as type:” drop-down menu, select “dicomdir”
    d. Select “Save.”

Export files within the Exam: SaveAs

Note: if sonographers wish to conduct data transfer via this mechanism, a folder on the USB with the participant ID will need to be created on the USB prior to the scan.

1. Unlock the USB key with the key code/ password and insert it into the drive on the side of the ultrasound machine. A blue light will flash on the side of the key to acknowledge that the device has been activated.

2. Select the image(s) and/or cineloop(s) you wish to transfer.

3. Click the “Save As” icon on the lower left side of the screen.

4. The Save As screen will appear.
    a. From the “Save in archive:” drop-down menu, select the USB key.
    b. From the “Store” options, select the “Image Only” radio button.
    c. From the “Compression:” drop-down menu, select “JPEG”
    d. From the “Quality” drop-down menu, select “100”
    e. From the “Save as type” drop-down menu, select “Raw Dicom”
    f. Select “Transfer.”

Eject the USB Key

1. Push “F3” on the keyboard.

2. Select “Stop Device” on the dialog box.

3. Select “OK” once the device can be safely removed.