Title of Study: Alternating Urine Redox Status of Consecutive Menstrual Cycles [Uro-001]

Sponsor: Urobiologics LLC, 31628 Glendale St., Livonia MI 48150

Date of Final Protocol: October 16, 2019

Approved by:

For the Sponsor:

[*Signature on File*]

10/16/19

Kuldeep Verma, PhD
President, Urobiologics, LLC

Principal Investigator:

[*Signature on File*]

10/16/19

Dr. Daniel S. McConnell, PhD
Associate Research Scientist, Department of Epidemiology
University of Michigan School of Public Health

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Alternating Urine Redox Status of Consecutive Menstrual Cycles [Uro-001]

Name of Sponsor:
UROBIOLOGICS, LLC

Population:
40 Female subjects

Estimated duration of study:
November 2019 – October 2020

Objective:
The Sponsor believes there is variability in the relative redox status in women's urine across menstrual cycles. This validation study will involve the collection and analysis of urine samples from non-pregnant women to determine the existence of alternating redox status between two consecutive menstrual cycles. If the hypothesis is proved, future research could examine if there are related fertility issues.

Study design synopsis:
Approximately 40 women with normal reported menstrual cycles will be included in this validation study.

All subjects will receive an explanation of the study and what is asked of them. They will sign informed consent and then undergo screening for study eligibility. If subjects fail the screening evaluation for reasons related to incidental transitory conditions yet wish to participate, they may subsequently be re-screened again after providing informed consent.

Subjects who meet the entry criteria (along with providing informed consent) will receive written instructions for participation in the study; an information sheet for logging their period dates, tracking medications and fevers, and instructions for returning the samples; and all supplies needed for collecting the urine samples (collection kits).

Subjects will collect urine over two days at the mid-point of their menstrual cycles (see Appendix 1) until three separate pairs of two consecutive cycles are obtained (requiring a minimum of 6 months of participation in the study). Subjects may receive telephone calls, texts, or other reminders to obtain the urine, as long as the reminder method is acceptable to the subject.

Urine specimens will be delivered to the University of Michigan CLASS Laboratory or to a second location for transfer to the CLASS Laboratory; if necessary, specimens may be sent through postal or carrier services. In the CLASS Laboratory, aliquots of urine will be drawn from the specimens, randomized and blinded in consecutive cycle pairs, and sent to Urobiologics for analysis. Urobiologics will send the reports of results to the CLASS Laboratory.

When all samples have been collected and analyzed, the blind will be broken, the data coded into spreadsheets, and statistical analysis will be performed. The principal hypothesis is that women have menstrual cycles that alternate between opposite redox states.

Inclusion/Exclusion Criteria:

Inclusion criteria:

1. Female subject, between the years 18-30, at the time of consent.
2. Subject reports "regular" menstrual cycles (i.e., cycle is 25 to 35 days and subject can typically predict onset of menses within ± 3 days).
3. Subject may be sexually abstinent or sexually active; if sexually active, she should be trying to prevent pregnancy using non-hormonal methods such as condoms.
4. Subject is willing to participate and is capable of giving informed consent. (Note: Consent must be obtained prior to any study-related procedures.)
5. Subject must be willing to comply with all study procedures.
6. Subject agrees to avoid alcohol, unapproved medications (prescription, OTC, or other), marijuana products, and illicit substances for 3 days prior to each urine collection. If medications must be taken, the collection will be discarded and another opportunity offered.

Exclusion criteria:

1. Subject is pregnant, or trying to or planning to become pregnant during the study.
2. Subject was pregnant within 1 year of beginning study, regardless of the outcome of pregnancy.
3. Subject who is breastfeeding.
4. Subject who is not menstruating for any reason or plans to undergo or change circumstances that might affect menstruation (e.g., certain athletic training regimens, significant dietary changes).
5. History of recent or current irregular menstrual cycles (as self-reported by the subject).
6. Subject reports she has been told by a medical professional that she is not ovulating (i.e., has anovulatory periods) at any time during the previous 12 months.
7. Subject is using or planning to use any method to regulate her periods.
8. Subject smokes (includes tobacco and marijuana) or vapes.
9. By history, subject has medical problems that might influence the results of the study, such as endocrinologic, renal, or hormonal problems.
10. Known polycystic ovarian syndrome (by history).
11. Subject is taking or planning to take hormones or hormone- enhancing/manipulating pharmaceuticals (including "morning after pill" [levonorgestrel], birth control pills, Clomid [clomiphene citrate], Femara [letrozole], Cialis [tadalafil]*, Viagra [sildenafil]* and similar medications) and/or agents to influence hormonal status such as herbal products or nutritional supplements. (*Not approved for use and should be avoided by women.)
12. Subject is using or planning to use hormonal contraceptives of any type, including oral contraceptives and intrauterine devices.
13. Subject is using or planning to use hormonal therapies for acne vulgaris or other conditions, including spironolactone, flutamide, metformin, or oral contraceptives used as acne therapies, including Ortho Tri-Cyclen (combination of norgestimate and ethinyl estradiol [EE]), Estrostep (combination of norethindrone acetate and EE), and Yaz/Yasmin (combination of drospirenone and EE).

14. Subject is using or planning to use estrogen products, including topicals/creams, for any purpose.
15. Subject is using or planning to use androgens or anabolic steroids (e.g., stanazolol/danazol) for any reason, including for training or athletic purposes..
16. Subject has attempted or taken IVF or IUI treatment within the prior six months.
17. Subject has uncontrolled diabetes or other significant medical disorder that might, in the investigator's opinion, preclude completion of the study or suggest that an exclusion criterion will ensue.
18. Subject reports difficulty handling biologic fluids, specifically urine, or gives other reasons why she is hesitant to participate.

Methods for subjects to collect urine samples:

See Appendix 1 for instructions to subjects and methodology.

Delivery of samples to UM research laboratory:

See Appendix 1.

Processing of samples in UM research laboratory:

Preparation of samples:

1. Upon receipt of the sample collection kit from each subject, check to ensure that the bag label has been transferred to the Sample Collection Form. Verify that the urine barcode numbers listed on the sample sheet match the numbers on the urine vial. Assure that two separate 30 mL vials are present from the same subject, one labeled "A" or "#1" and one labeled "B" or "#2", AND that they contain dates consistent with being obtained during two consecutive menstrual cycles (i.e., about 25-35 days difference), AND that the subject so states on the form filled out by the subject. A difference in days outside the range of 25 to 35 days could legitimately occur, but if the gap is not within 25-35 days, ask Dr McConnell to investigate.
2. Indicate date of receipt of samples on the Sample Collection Form.
3. Obtain a ACDC Aliquot Kit. Transfer the LG Bag Label to the Sample Collection Form in the space below the Subject ID, Date, and Time.
4. Invert repeatedly to ensure homogeneity.
5. Carefully harvest the urine from the 30 mL "A or #1" vial and pipet 10 mL into one yellow-top polypropylene 10 mL tube labeled with the predetermined randomization number. Tube will also be labeled "Biologic research specimen. Human urine."
6. Cap and place the 10 mL vial in its corresponding numbered box.
7. Repeat steps 4-6 for the 30 mL "B or #2" vial.
8. Write the date, time and the technologist's initials on the Sample Collection Form. Also include the tube numbers, volume and number of tubes.

9. Place the box containing the 30 mL vial and the randomized yellow-top tubes into the room temperature ACDC storage cabinet.
10. File the Sample Collection Form in the ACDC sample binder.

Quality assurance (QA):

Under the direction of the study statistician, at other times, samples may be aliquoted from the 30 mL vials and given randomization numbers (or facsimiles thereof) by following steps 4-6 above. Such specimens will come from subjects' consecutive cycles but may not necessarily be paired in the same way as the principal samples. For example, if previous randomized samples were from Subject X in months 1&2 and 3&4, then QA could include sending samples from Subject X's months 2&3 and/or repeat samples from the original months such as 3&4. Such QA may be performed and analyzed at interim stages of the protocol only if it does not break the blind of the principal study. Repeated failure to be in conformation with the hypothesis during QA may result in early termination of the study, if the statistician and principal investigator agree.

Shipping of samples:

When sufficient samples are obtained, the 10 mL yellow-top tubes labeled "Biologic research specimen. Human urine." with random numbers affixed AND NO OTHER INFORMATION AFFIXED (specifically excluded is any information that could in any way identify the participating subject by name, birthdate, or any other identifying information) will be sent (by mail or other approved delivery method) for processing to Urobiologics LLC, 31628 Glendale St., Livonia MI 48150. THE GREEN-TOP VIALS THAT SUBJECTS USED FOR THEIR COLLECTION OF URINE MUST REMAIN LOCKED IN THE C.L.A.S.S. LABORATORY AND MAY NOT BE SHIPPED OUT (because they may contain identifying information about the subjects or might otherwise "break the blind").

WHEN THE SUBJECT BELIEVES THAT SHE WILL LIKELY SHIP HER SPECIMENS BACK AND NOT RETURN SAMPLES IN PERSON, THE STUDY WILL PROVIDE THE APPROPRIATE MATERIALS TO SHIP THE SPECIMEN THAT COMPLY WITH ALL DOT REGULATIONS. THE STUDY WILL PROVIDE THE TRIPLE PACKAGING (1- VIAL, 2 SEALED BAG, 3- ENVELOPE) RETURN ENVELOPES WITH PREPAID RETURN SHIPPING LABEL. THESE URINE SAMPLES ARE CONSIDERED INFECTIOUS SUBSTANCES CATEGORY B (SEE NOTE BELOW), THEREFORE TO COMPLY WITH DOT REGULATIONS THE SAMPLE MUST CONTAIN THE TEXT: "EXEMPT HUMAN SPECIMEN" WHICH MUST BE VISIBLE ON OUTSIDE OF ENVELOPE. THIS EXEMPT STATUS IS FOR SPECIMEN SHIPPING ONLY AND DOES NOT IMPLY ANY IRB EXEMPTION.

URINE IS CONSIDERED UNDER **INFECTIOUS SUBSTANCES CATEGORY B**

MATERIALS THAT ARE INFECTIOUS BUT DO NOT MEET THE CRITERIA FOR INCLUSION IN CATEGORY A
ASSIGNED TO UN 3373 (CATEGORY B, INFECTIOUS SUBSTANCES)
HUMAN OR ANIMAL MATERIAL TRANSPORTED FOR RESEARCH, DIAGNOSIS, DISEASE TREATMENT, ETC.

PATIENT SPECIMENS FOR WHICH THERE IS MINIMAL LIKELIHOOD THAT PATHOGENS ARE PRESENT AND ARE TRANSPORTED IN A PACKAGING WHICH

WILL PREVENT ANY LEAKAGE ARE CONSIDERED **"EXEMPT HUMAN SPECIMEN"** OR **"EXEMPT ANIMAL SPECIMEN"**.

THEY MUST BE LABELED AS "EXEMPT HUMAN/ANIMAL SPECIMEN".
THEY MUST USE TRIPLE PACKAGING SYSTEM.

Processing of samples in Urobiologics laboratory:

Urine samples are processed to remove non-hormonal redox compounds and then reacted with a redox indicator to evaluate each sample in the pair of urines as an oxidative or a reductive reaction.

Results are reported to the UM C.L.A.S.S. Laboratory according to the randomized numbers provided with the samples.

At the conclusion of the study the residual de-identified urine will be shipped to the Sponsor. Upon completion of processing, the sponsor will destroy all remaining materials and verify the destruction and disposition to the class laboratory.

Statistical power analysis and planned stat analysis:

From each experimental subject, we will have three non-overlapping pairs of consecutive urine samples taken. For each sample pair a blinded determination will be made by Urobiologics as to which sample in the pair was reductive. For the sake of simplicity, consider the case where these samples are collected in 6 consecutive months. Let "A" be the event that the reductive sample in the pair corresponds to the earlier cycle and "B" its converse. Then, the hypothesis of alternating reductive cycles is supported if the sequences AAA or BBB are over-represented relative to others. To analyze this, we will map whether the 2nd and 3rd pairs are successfully determined conditional

on the first. Let "Y" denote success and "N" denote failure. Each subject then has one of the four patterns: YY, YN, NY, NN representing whether the 2nd and 3rd paired cycles are correctly predicted by the alternating cycle hypothesis. The hypothesis is supported by over-representation of YY relative to chance if predictions were made under the null of P(Y) = 0.5 with successive pairs called independently.

Consequently, we will first use a chi-squared goodness of fit test to evaluate the hypothesis below at the α=0.01 level:

H_0: p = 0.5

and P(YY) = p*p; P(YN) = P(NY) = p*(1-p); P(NN) = (1-p)*(1-p).

Under the alternative that p = 0.75, 37 subjects are needed to give 90% power to reject the null hypothesis at a significance level of 0.01. Assuming that <10% ofenrolled subjects will be lost to attrition, we need to enroll a minimum of 40 subjects to achieve the target sample size.

Subsequently, we will estimate "p" in the model above using the total proportion of "Y" and form a confidence interval for this estimate using a bootstrap procedure to account for the pairing of samples within women. As a secondary analysis, we will use a likelihood ratio test to evaluate the validity of the independence assumption by comparing the one-parameter independence modelto the four-parameter model with unique cell probabilities. Finally, for exploratory analyses to generate future hypotheses we will fit a mixed logistic regression in which the log odds of outcome "Y" are expressed as a linear function of any covariates to be explored and a random intercept inducing correlation between repeated measures within each woman.

Study Flow Chart

Study Procedure	Screening	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	All samples processed
Informed Consent	X							
Inclusion/Exclusion form	X							
Medical History/ Demographics	X							
Assign a Subject Number	X							
Give three urine collection kits to subject. Each *kit* will contain two **units**. The two **units** provide for two collections about a month apart for each *kit*. Each **unit** will have one cup, one green-cap vial, one dropper, one instruction sheet, and one form to fill out with period start date and dates of collection of sample along with other relevant queries and instructions for returning the samples. When the subject believes THAT she will likely ship her specimens back and not return samples in person, THE STUDY WILL PROVIDE THE APPROPRIATE MATERIALS TO SHIP THE SPECIMEN THAT COMPLY WITH ALL D.O.T. REGULATIONS. THE STUDY WILL PROVIDE THE TRIPLE PACKAGING (1- VIAL, 2 SEALED BAG, 3- ENVELOPE) RETURN ENVELOPES WITH PREPAID RETURN SHIPPING LABEL. THESE URINE SAMPLES ARE CONSIDERED INFECTIOUS SUBSTANCES CATEGORY B (SEE NOTE BELOW), THEREFORE TO COMPLY WITH D.O.T. REGULATIONS THE SAMPLE MUST CONTAIN THE TEXT: “EXEMPT HUMAN SPECIMEN” WHICH MUST BE VISIBLE ON OUTSIDE OF ENVELOPE. THIS EXEMPT STATUS IS FOR SPECIMEN SHIPPING ONLY AND DOES NOT IMPLY ANY IRB EXEMPTION.	X*							
Determine if subject accepts reminders	X							
Remind subjects at least twice in each cycle (when and as agreed by subject)		X	X	X	X	X	X	

Receive urine samples, process in CLASS Lab per protocol.			X		X		X	
Send urine samples to Urobiologics			IN BATCHES OF SAMPLES					
Code data from Urobiologics				AS SAMPLES ARE REPORTED				
Quality assurance per protocol			AS SAMPLES ARE AVAILABLE					
Break blind; statistical analysis								X

*At any time, provide more kits if needed.

APPENDIX 1: Instructions to subjects:

HOW TO COLLECT URINE SAMPLES

This study may help future women with fertility issues. Because this topic is so important, please follow the directions carefully. Call us with any questions at (734) 763-2461.

It is very important that samples be collected over two nights during consecutive periods -- otherwise the result may be confusing or misleading.

If you make a mistake, call us and we will see if we can fix it together. If you collect urine in one period and forget to collect in the next cycle, please let us know right away. Again, we will work with you to get enough pairs of urine collections to complete the study. If you don't tell us, the entire study could be ruined for everyone.

If you develop fever, flu, or a virus at the time of collection, make a note of it on the sheet we gave you. If you can get your actual temperature, please write it down. Give us a call during business hours and we will advise you if you should reschedule your urine collection.

If you are taking some regular medicines, it would be better to stop taking them 2 days before and resume them after collecting the overnight samples. Please consult with your doctor about this and get permission to stop for two days. If you can avoid unnecessary doctor visits during the collection time, it is best because you may receive a treatment that affects the research test. Of course, if you need to see a doctor, please do so. Record any medications, drugs, over-the-counter products, etc. that you are taking on the sheet provided.

To assess our research theory, we need overnight mid-cycle urine samples from two nights to be combined into one vial during your period. Then we need this repeated in your very next period. In each period, you collect your urine overnight for any two nights from the 13th to 17th night after the START of your period. The start of your period is the first day of bright red bleeding. Earlier spotting is not considered the first day.

(Example: Suppose your first day of bright red bleeding occurs on Jan 5th. You will collect your urine on any two nights from Jan 18, 19, 20, 21, and 22 – that is, 13 to 17 nights after the first day. If you collected on the 13th night but forget to collect on the 14th night, you can still collect the second night on the 15th, 16th, or 17th night. It is any **two** nights during the 13th to 17th night following the START of your period.)

SUMMARY:

At the mid-point of your period, collect all urine over 2 different nights. Collect all urine *after* you go to bed including the first urine of the morning. Put a small amount of each urine collection into the green-top vial. If necessary, top off the green-top vial. *Repeat in your next period*. The two green-top vials are what we need for our research.

How do I collect the urine over 2 different nights on days 13 to 17 of my period?

First, set out on the back of your toilet (or nearby) the large plastic collection cup, the dropper, and the resealable green-top vial.

FOR THESE FIRST CYCLE COLLECTIONS, THE GREEN-TOP VIAL **MUST** BE LABELED "**#1**".

We need your urine for the entire night. Before going to bed, pass urine into the toilet. Do not collect this -- flush it away. Collect all urine every time you go to the bathroom after this, *including* your urine when you get up in the morning.

The way to do this is to:

1. Pee (urinate) into the toilet for 2 to 3 seconds
2. Collect the rest of the urine in the provided plastic collection cup (called "midstream" urine).
3. Then transfer urine from the cup to the resealable green-top vial, using the dropper provided. Each time you pee, put some of the urine into the green-top vial as follows:

If you wake up during the night, collect any midstream urine in the collection cup.

4. Take two dropperfuls (total about 6 ml or a bit more than a teaspoon) of the urine from the cup and squirt the dropperfuls into the green-top vial provided. You do not have to be exact.
5. Close the vial.
6. Empty the extra urine left in the collection cup into the toilet and flush the urine away.

When you get up in the morning, collect midstream urine in the collection cup.

7. Again take two dropperfuls from the cup and squirt them into the same green-top vial.
8. If you did not urinate during the night, you will have collected only your morning urine in the cup so put FOUR dropperfuls into the green-top vial.
9. Close the vial.
10. Empty the extra urine left in the collection cup into the toilet and flush the urine away.
11. If you wish, rinse the collection cup with tap water and allow it to dry.
12. Keep the materials at hand for the next night's collection.

The green-top vial should be about half-full after the first collection night. Make sure the cap is on securely and the vial will be leak proof.

Your morning urine is your last collected sample for the day. Do not collect any samples during the day.

Repeat during the next night (or, if necessary, another night within the 13 to 17 day window of this period). Again, put the materials you need nearby, including the green-top vial **#1**. When you go to bed, flush away the urine you pee. Collect any urine during the

night and when you get up in the morning. Add dropperfuls of urine to the **SAME** green-top vial in the same way as above.

With two nights' collection, you will have approximately 30ml of urine in the vial. The vial will be nearly full (near the 30ml mark on the vial). If you have put in less than that amount, you can make it up by adding some more urine from the last sample. After that, you may throw away the dropper and the collection cup. **TIGHTEN THE CAP ON THE GREEN-TOP VIAL AND KEEP IT. You will not be adding anything more to it.**

Your first menstrual cycle sample is ready! Please be sure you have entered the date of your period's first day and the dates of urine collection on the sheet provided.

Store the green-top vial labelled "#1" at room temperature until after the next collections in your next period. You may put a "Do not open" Post-it note on the cap or similar to remind you not to add anything else to this vial. Your urine samples won't go bad at room temperature. No preservative is needed.

Please keep your completed green-top vial, these instructions, the sheet you are filling out, and the remaining collection items where you will find them during your next period! As a reminder, you can find these instructions attached to the Consent Form you signed. Call us during business hours at (734) 763-2461 if you need more collection materials or if you have ANY questions.

How do I collect the urine over 2 different nights on days 13 to 17 of my next period?

In your next period, repeat the process. Collect your urine in the same way as above. BUT THIS TIME PUT THE DROPPERFULS OF URINE in the second green-top vial LABELLED "**#2**". Finish filling out the information sheet. Now you can hand-deliver or ship both green-top vials ("#1" and "#2") and your information sheet all together to one of our University of Michigan campus offices. Instructions are in the sheet provided.

We hope you will participate for a total of 6 menstrual cycles (3 times with 2 consecutive periods). Thank you for your help with our research. We appreciate your effort!