IRB Protocol #1, version #2
April 1st 2022

**Platelet-Rich Plasma Injection for the Treatment of Stress Urinary Incontinence in Females: A Randomized Placebo-Controlled Study**

**Specific aims**
The primary aims of this randomized controlled trial study are to:

1. Evaluate the efficacy of platelet-rich plasma (PRP) injection into the anterior vaginal wall for the treatment of stress urinary incontinence (SUI) compared to placebo.
2. Evaluate the safety of PRP injection into the anterior vaginal wall for the treatment of SUI.

**Hypothesis**
Anterior vaginal wall PRP injection will be safe and associated with improved SUI compared to placebo.

**Research Strategy**

**Significance and Innovation**
Stress urinary incontinence (SUI), defined by the International Continence Society as the involuntary leakage of urine on effort or exertion, or on sneezing or coughing [1], is estimated to affect up to 49% of women [2, 3], many of whom undergo an operative intervention. Conservative management for SUI includes lifestyle interventions, pelvic floor muscle training, and incontinence pessaries. If conservative treatment fails or is not desired, surgical treatment via midurethral sling, colposuspension, pubovaginal sling, or bulking agent may be considered. Although surgery is highly effective in curing SUI, there are significant risks, such as hemorrhage, visceral injury, voiding dysfunction, chronic pain, and mesh exposure. Specifically, mid-urethral sling-related complications have been reported in up to 6% of patients [4] and include pain, exposure, erosion, dyspareunia, and long-term disability [5]. Complications after urethral bulking can include abscess formation, delayed hypersensitivity reactions, and vaginal erosion [6]. Fascial slings, compared to mid-urethral slings, have been shown to have a higher incidence of voiding dysfunction sometimes requiring intermittent catheterization, urethrolysis and/or prolonged suprapubic catheter use [7]. **Thus, there is an urgent need to evaluate innovative, minimally invasive, and low-risk procedures for SUI.**

Platelet-rich plasma (PRP) is an autologous solution of human plasma with a high concentration of platelets. Platelet-rich plasma contains various growth factors that enhance the healing process and regeneration by initiating the stages of cell proliferation, differentiation, angiogenesis, and chemotaxis. The rationale for use and therapeutic potential of a high concentration of platelets is based on their capacity to supply supraphysiologic amounts of essential growth factors and cytokines which then provide a regenerative stimulus, promoting tissue repair [8]. Important growth factors released from platelets during the healing process include platelet-derived growth factor (PDGF), transforming growth factor (TGF-β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF-1) [9].

PRP has been described for the treatment of various health conditions, from hair loss to joint injuries. PRP is most studied and used for the treatment of musculoskeletal soft tissue injuries, including tendinopathies and tendon tears, and ligament, muscle, and cartilage injuries [10]. The injection of PRP is not a new technology, but it has not been well-studied in the field of urogynecology. Two small series have reported on use of PRP

for the treatment of female SUI, suggesting successful treatment outcomes in up to 80% of patients [11] [12]. With this study, we will provide higher quality evidence on the use of PRP in the treatment of SUI.

**This study will aim to determine the 6-month post-treatment efficacy of PRP injection in the treatment of SUI.** Knowledge gained from this study has the power to change clinical practice by equipping physicians with a minimally invasive and likely low risk SUI treatment.

**Approach**

The primary aims of this study are to determine the efficacy and safety of PRP injection into the anterior vaginal wall for the treatment of SUI.

The primary efficacy aim will be determined by the following outcome measures:

1. Negative urinary stress test (no leakage noted on examination during cough or Valsalva maneuvers at a standardized bladder volume of 300 mL [13]
2. Subjective outcome with improvement in the Patient Global Impression of Improvement (PGI-I), with positive response defined as "very much better" (1) or "much better" (2). [14]

Treatment "success" will be defined as a negative CST and a reported PGI-I of "very much better" or "much better" 6 months after treatment.

The primary safety aim will be assessed by monitoring adverse events classified by the Clavien-Dindo Classification.

Secondary Outcomes that will be compared between groups include:

1. Female Sexual Function Index (FSFI) scores
2. Incontinence-Quality of Life (I-QOL) scores
3. Perception of Monetary Value
4. Questionnaire for Urinary Incontinence Diagnosis (QUID)
5. Visual Analog Scale (VAS) for patient pain/discomfort
6. Visual Analog Scale (VAS) for procedure difficulty (by provider)

**Eligibility Criteria**

1. Women aged 18 years or older
2. Pure or stress-predominant urinary incontinence with the Medical, Epidemiologic, and Social Aspects of Aging questionnaire (MESA) stress incontinence symptom score greater than MESA urge incontinence symptom score
3. Observation of leakage by provocative stress test at bladder volume $\leq$ 300 mL [15]
4. Post void residual < 150 mL

**Exclusion criteria**

1. Currently pregnant or trying to conceive
2. Currently breastfeeding
3. Interstitial cystitis

4. Urgency urinary incontinence predominance or currently being treated for overactive bladder with medication, percutaneous tibial nerve stimulation, bladder chemodenervation, or sacral neuromodulation
5. Currently being treated for a sexually transmitted disease
6. Pelvic organ prolapse greater than stage 2 according to the Pelvic Organ Prolapse Quantification System
7. Periurethral mass
8. Active gynecologic, urologic or colorectal cancer
9. Any history of cancer within the last 5 years
10. Mental disorder making the patient unable to provide consent
11. Undiagnosed abnormal uterine bleeding
12. Genitourinary fistula
13. Prior SUI surgery
14. Use of anti-platelet or anti-coagulant medication
15. Use of non-steroidal anti-inflammatories
16. Regular use of non-steroidal anti-inflammatories

**Methodology**

This is a single-blind prospective randomized controlled trial comparing the efficacy of injection of 5 mL PRP into the anterior vaginal wall versus injection of 5 mL injectable saline for the treatment of SUI in females.

The study population is women aged 18 years or older with stress-predominant urinary incontinence conformed by a positive cough stress test, as well as a higher SUI MESA score over the UUI MESA score. The approximate number of subjects will be 50, based on a power analysis as described below. The length of follow up will be 6 months post injection.

Following approval by the local Institutional Review Board (IRB), subjects will be recruited from the University of Iowa Hospitals and Clinics Urology and Urogynecology Clinics during regularly scheduled appointments. Subjects will be referred to the study by physician referral. This trial will be registered with clinicaltrials.gov prior to any subject enrollment.

**Enrollment**

Potential participants will be approached in clinic and be given an overview of the study. If they meet the inclusion criteria and none of the exclusion criteria, the research coordinator will initiate the informed consent process. To assure patient privacy during the informed consent process, the discussion will be held in a private room at the clinic, with the door closed. Once the potential participant has ample time to consider the study, they will be asked to sign the written consent form with the consent provider. A copy of the signed consent form will be given to the patient and the original placed in the research record.

**Equipment**

Using the Arthrex Autologous Conditioned Plasma (ACP) Double Syringe System (Arthrex Inc, Naples, Florida), approximately 15 mL of the subject's venous blood will be collected from the antecubital fossa. The sample, in the ACP system, will be centrifuged in the clinic lab space. A representative from the company may be present for this portion of the procedure to ensure proper use of the centrifuge. However, the company is not funding this study, has not been involved with protocol development and will not be involved in data analysis,

interpretation or publication of results. Centrifuging takes approximately 5 minutes. At the end of the process, approximately 5 mL of the subject's PRP will be separated. The principal investigator (PI) and research staff will be trained on the processing procedure by Dr. Ryan Kruse from the Sport Medicine Clinic and by the company representative, in order to ensure the procedure is performed using standardized and safe methods.

**Randomization**

Randomization will be performed with the help of a biostatistician. Participants will be block randomized with equal allocation to the treatment and control groups. To develop the assignment table, blocks of size 2, 4, or 6 participants were randomly selected until we achieved the anticipated sample of 50. Within each block, half of participants will be randomly assigned to one group and half to the other, ensuring equal treatment allocation. The randomization schedule will be transferred to sequentially numbered, opaque, sealed envelopes.

The principal investigator and the research coordinator will be responsible for maintaining the confidentiality and security of the randomization.

At this point, the clinical research coordinator will then open the randomization envelope. Randomization will be performed as described. The subject's initials and the date of injection will be written on the outside of the envelope prior to the envelope being opened. This writing will be transferred to the card containing the assigned randomization inside the envelope with carbon transfer paper. If the subject is assigned the active treatment, the sample will be used. If the subject is assigned the placebo, the sample will be discarded appropriately.

**Injection Procedure**

The injection procedure will be performed using previously published methods. The PI and 1 co-investigator will perform the procedure to ensure it is done in a standardized and reproducible method throughout the trial.

The injector will enter the clinic room with the patient already in the dorsal lithotomy position. A local anesthetic of lidocaine gel 2% will have been applied to the distal anterior vaginal wall 20 minutes before injections. The injector will then inject 5 mL total of either the active PRP or the placebo based on randomization. The PRP will be injected into 3 sites of the anterior vaginal mucosa at the mid-urethra (about 1 cm proximal to the urethral meatus) using a 30-gauge needle. *Figure 1* diagrams the location and depth of the injections. The location of the injections is based on a pilot study by Long *et al* [12]. Immediately after the procedure, the patient will be given the VAS for pain/discomfort related to the procedure and the injector will complete the VAS for procedure difficulty.

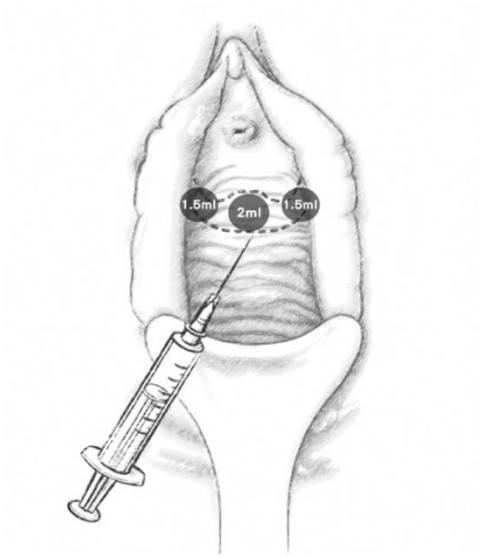

*Figure 1. Location and Depth of Injections*[12]

At baseline and at time points of 1 month, 3 months, and 6 months, subjects will complete the previously described validated questionnaires (see visit details below). At a 1-month telephone visit, a subset of the questionnaires will be administered, and patients will be queried about any adverse events. At baseline and at time points of 3 and 6 months, all validated questionnaires will be completed, as well as a pelvic exam with a urinary stress test, performed by a nurse practitioner specifically trained in urogynecology, and who did not perform the injections and thus blinded to the randomization. All adverse events will be recorded and classified per the Clavien-Dindo Classification.

At the end of the 6 months, subjects will be unblinded.

If subjects have symptoms of SUI which are refractory to treatment with PRP they will be referred to Urology or Urogynecology and will be offered standard of care following completion of the final study visit. If patients wish to receive alternative treatment for SUI prior to completion of 6-month follow-up they will be exited from the trial and final outcomes will be assessed at that time

Subjects will be provided a $50 eVoucher upon completion of the 6-month follow-up visit.

Data will be stored in a secure REDCap database, only accessible to the research team members. Randomization envelopes and the Randomization Log will be kept in a locked cabinet.

**Power Analysis**

Sample size calculations were performed based on a desired power of at least 90% and assuming true response rates of 70% and 20% for treatment and placebo groups, respectively. The 70% response rate in the active PRP treatment group was chosen based on prior studies of PRP, as well as urethral bulking procedures [12] [16] [17]. The response rate of 20% in the placebo arm was chosen based on prior urethral bulking studies showing a 20% rate of negative CST after placebo [18]. Using an unadjusted alpha of 0.05, a sample of 38 subjects (19 per treatment group) is the minimum number required to have >90% power to detect a difference between the response rates of the two treatments.

In order to account for a 20% drop-out rate, we will plan to enroll a total of 50 patients (25 per treatment group).

**Statistical Analysis and Data Reporting**
For this study we will assess the demographics of our patients by providing summary statistics stratified by treatment group. Comparisons between the demographics of the two treatment groups will be made using Fisher's Exact or chi squared tests for categorical variables and using T-test or Wilcoxon rank sum tests for continuous variables, where appropriate. Primary and secondary outcomes will also be compared using treatment-stratified summary statistics, and p-values obtained using the aforementioned statistical tests.

Univariate logistic regression models will be fit using the demographic variables obtained in the study, as well as treatment, to predict patients' primary outcomes. Then, one multivariate model will be constructed for each primary outcome with treatment included as a predictor and an AIC-based stepwise forward model selection procedure dictating what other variables to include into the model. Univariate generalized linear models using treatment group as the predictor variable will also be examined for the secondary outcomes.

Data will be reported using the CONsolidated Standards Of Reporting Trials (CONSORT) guidelines and standard flow diagram.

**Breaking the Blind**
A subject's treatment assignment will not be broken until the end of the study unless medical treatment of the subject is dependent on knowing the treatment the subject received. Should a medical emergency require the blind to be broken the investigator may unblind an individual subject's treatment allocation.

**Research Visit Summary:**
**Baseline visit confirming eligibility with inclusion and exclusion criteria**
Basic demographic data will be recorded
Past medical history will be recorded
Pregnancy test (if of child-bearing age)
Urinalysis dipstick to exclude active urinary tract infection
Bladder scan to see how much urine is in the bladder prior to urinary stress test
Pelvic exam with urinary stress test
Bladder scan after voiding to check post-void residual
Three surveys before the procedure

- Female Sexual Function Index
- Incontinence Quality of Life
- Questionnaire for Urinary Incontinence Diagnosis

Blood draw of about 15 mL
Randomization
Injection procedure
Visual analog Scale (VAS) for pain/discomfort associated with the procedure
Injector completes Visual analog Scale (VAS) for level of difficulty of procedure

**1-Month Telephone Visit**
Record any adverse events

Surveys

- Incontinence Quality of Life
- Questionnaire for Urinary Incontinence Diagnosis
- Patient Global Impression of Improvement

**3-Month Visit**

Record any adverse events
Pelvic exam with cough stress test
Surveys

- Female Sexual Function Index
- Incontinence Quality of Life
- Questionnaire for Urinary Incontinence Diagnosis
- Patient Global Impression of Improvement

**6-Month Visit**

Record any adverse events
Pelvic exam with cough stress test
Surveys

- Female Sexual Function Index
- Incontinence Quality of Life
- Questionnaire for Urinary Incontinence Diagnosis
- Patient Global Impression of Improvement
- Perception of Monetary Value

$50 eVoucher for completion of 6-month follow-up visit

**Benchmarks for Success**

After enrollment of the first 5 subjects, a quality control of the data collected and entry will be performed. Additional quality control will be performed by the PI after every 10 patients. Meticulous recording of adverse events will be performed. If adverse events are frequent and distressing, the project will be halted and data collected to that point will be analyzed.

After enrollment of 25 subjects, preliminary data and adverse events will be analyzed by a Data and Safety Monitoring Board made up of the research team members.

**Potential Problems**

Potential problems include low or slow enrollment. In addition to screening patients in the clinic, we can also plan on advertising the study via the University of Iowa listserv and/or advertisement materials to other clinics, such a family medicine, internal medicine, and gynecology.

Since this is a new procedure not well-described in the literature, another potential problem may be the learning curve of the procedure. To mitigate this, only two clinicians will serve as the injector, including the PI

and one other fellowship-trained FPMRS specialist. This will allow consistent and standardized procedure methods.

**Timetable and Plan For Completing Project**

February 2022: set up REDCap database
March 2022: complete IRB application (as of grant submission, the IRB application has been approved pending minor modifications)
June 2022: start screening clinics for eligible patients, enroll subjects
June 2022 – September 2023: enroll patients, collect data

Our urology and urogynecology clinics see approximately 120 female SUI patients/month. Approximately 40 of these are likely to be eligible for enrollment. Assuming an enrollment rate of 10%, this would allow about 4 patients to be enrolled/month Thus, we plan to meet our total enrollment goal of 50 patients (40 patients + 10 to account for a 20% dropout rate) by about 15 months.

March 2023: complete enrollment of subjects, complete 6-month follow-up
April 2024: start data analysis
May 2024: complete data analysis, start manuscript writing
July 2024: complete manuscript writing

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