Metabolic and neurobehavioral response following intraovarian administration of autologous activated platelet rich plasma: First qualitative data

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Abstract

OBJECTIVES: This work assessed sexual and neurobehavioral parameters after ovarian treatment with autologous PRP.

DESIGN: Questionnaire study.

MATERIAL AND METHODS: Patients receiving ovarian PRP injection (n=80) due to low ovarian reserve and/or at least 1 prior failed IVF cycle were sampled. Pre- and post-treatment levels in self-reported daily energy, sleep quality, skin tone/hair thickness/nail growth, cognitive clarity, menstrual pattern, cervical mucus/vaginal lubrication, libido, sexual activity, ability to achieve orgasm, and overall sexual experience were measured.

RESULTS: Mean±SD age and baseline BMI among patients were 45.5±6yrs and 25±5.1kg/m², respectively. Average weight loss after ovarian PRP was 1kg (p=0.028). After ovarian PRP, superior nail growth, skin tone, and hair thickness was observed by 46.3% of patients [95%CI=35%,57.8%]; the same ratio experienced increased “clarity of thinking” following the procedure. Irregular or absent menses affected 56.3% of patients at enrollment, and menses returned or cyclicity improved in 24.4% after treatment [95%CI=12.9%,39.5%]. Increased post-treatment vaginal lubrication/cervical mucus production was reported by 51.3% of women [95%CI=39.8%, 62.6%] accompanied by increased libido in 55% [95%CI=43.5%,66.2%]. More frequent sexual activity after ovarian PRP was noted from 46.3% of subjects [95%CI=35%, 57.8%] coinciding with a 45% improvement in overall sexual experience before vs. after ovarian PRP [95%CI=33.9%, 56.5%].

CONCLUSION: This investigation is the first to document responses across neurobehavioral and metabolic parameters after ovarian PRP. Injection of PRP-derived growth factors directly into ovarian tissue seems to enable a local signaling milieu favoring development of hormonally active ovarian elements, thus “re-potentiating” low or absent reserve.
INTRODUCTION

Platelets and their products (i.e., platelet rich plasma, PRP) have well-established roles in managing thrombocytopenia, yet PRP also comprises many soluble mediators critical to coordinate cellular repair after tissue injury (Nurden, 2011). Closely linked to inflammatory signaling, PRP also modulates tissue regeneration, cell proliferation and migration, extracellular matrix remodeling, programmed cell death, differentiation, and angiogenesis (Gurtner et al., 2008). Platelets are involved in the response to local tissue repair after capsular microtrauma in the adult human ovary after each ovulation, and likely contribute to overall organ function as well (Lacci & Dardik, 2010). Of note, the tissue regenerative effects of autologous PRP when applied to adult ovarian tissue have shown early promise in managing reduced reserve (Sfakianoudis et al., 2018). But independent of oocyte dynamics and IVF, what about broader, systemic responses following ovarian aging? Here we report on selected effects of ovarian treatment with autologous PRP using a questionnaire-based qualitative research model.

MATERIAL AND METHODS

A retrospective chart review was performed to identify women who received autologous PRP to one or both ovaries at a single center, designed as an assessment extension for subjects who enrolled in registered clinical trial NCT03178695 (U.S. NLM, 2017). In brief, dosing consisted of fresh isolation of substrate followed by injection of activated PRP into ovarian stroma, as previously described.[4] Ovarian PRP treatments were performed at a single center, by one clinician using uniform equipment for all cases.

Patient and public involvement

A multidisciplinary team developed a 30-item research questionnaire, derived from the standardized Female Sexual Function Index (Crisp et al., 2015) (see Table 1) Additional items were included based on redacted emails sent to clinic staff from patients after completing ovarian PRP. Queries were distributed by email invitation to those who underwent ovarian PRP procedure during a 16-month interval beginning April 2017. The questionnaire was configured electronically for secure internet access; there was no cost to participate and patients received nothing of value in exchange for contributing to the study. Participants were not able to access cumulative results until all data were received upon closure of the study. When the survey was pre-tested by volunteers (n=10), average time to answer all questions was approximately nine minutes. Incomplete questionnaires were not accessioned, and response origin IP addresses were monitored to block duplicate submissions. As this research component entailed no direct patient contact or collection of any identifiable personal health information, the study design was considered "no risk to human subjects"; additional IRB oversight was therefore not required.

Statistics

Chi-square test was used for equality of proportions. Maximum likelihood estimation (MLE) was used to determine proportions with 95% confidence interval to assure ≥95% coverage. P-values <0.05 were considered statistically significant. Dispersion of patient age was shown by boxplot among different groups (Frigge et al., 1989).

RESULTS

Valid email contacts were extracted from chart reviews for study patients who completed ovarian PRP during the assessment period (n=188). PRP treatment dates for participants are summarized in Figure 1. From these, full questionnaires were returned by 80 patients (43% response rate). In this sample, mean±SD patient age was 45.5±6 (range = 30.7-63.5, median = 45.1yrs) years. Mean±SD body mass index (BMI) for patients before vs. after PRP was 25±5.1 and 24.7±4.3kg/m2, respectively. Further analysis of patient weight following ovarian PRP found an average loss of 1kg among study patients (p=0.028).

In this sample, 45 women reported irregular or absent menses at baseline (56.3%); 11 of these (24.4%) observed return of menstruation or resumption of regular menses following ovarian PRP (p=0.041, by binomial proportion z-test). Among 46 women who reported some past or present HRT (hormone replacement therapy) use before enrollment, 31 were able to discontinue HRT following ovarian PRP treatment (67.4%; 95%CI=52%, 80.5%). There were 11 study patients who were not sexually active before undergoing ovarian PRP. In this subgroup, three women reported resumption of sexual activity after treatment (27.3%). Among patients who were sexually active prior to PRP (n=69), only three women reported negative change in sexual activity after ovarian PRP (4.4%). From these data, it was possible to evaluate reported improvement (27.3%) vs. impairment (4.4%) in sexual activity after ovarian PRP, and this difference was found to be highly significant (p=0.008, by N-1 χ² test).

When patients considered their daily average energy level, 45 of 80 patients (56.3%) reported reduced fatigue and beneficial improvement in energy level following ovarian PRP treatment [95%CI=44.7%, 67.3%]. Self-reports were also analyzed for patient observations regarding skin quality, nail growth, and scalp hair thickness/texture after ovarian PRP administration. For these parameters, 37 of 80 patients (46.3%) noted improvement after treatment [95%CI=35%, 57.8%]. Of note, this change following ovarian PRP treatment was found to correlate closely with daily average energy level (Pearson’s r=0.41; p<0.001).
We also sought to measure subjective change in cognitive acuity and mentation after ovarian PRP, and 37 of 80 patients (46.3%) noted increased “clarity of thinking” following the procedure [95%CI=35%, 57.8%]. This reported improvement in cognitive acuity was significantly correlated with both skin improvements (Pearson’s $r=0.36; p<0.01$) and energy level (Pearson’s $r=0.47; p<0.001$). Moreover, overall sleep quality among study subjects was reported to be better by 35 of 80 women (43.8%; 95%CI=32.7%, 55.3%) following ovarian PRP. This significant post-treatment change correlated significantly with skin improvements (Pearson’s $r=0.42; p<0.01$), increased energy level (Pearson’s $r=0.42; p<0.01$), and improved mentation (Pearson’s $r=0.39; p<0.01$).

After ovarian PRP, some study patients observed a substantial change in vaginal lubrication/cervical mucus production; 41 of 80 women indicated these factors had improved (51.3%; 95%CI=39.8%, 62.6%) with significant correlations measured with skin improve-
ments (Pearson's $r=0.32; p=0.004$), increased energy level (Pearson's $r=0.23; p=0.04$), improved mentation (Pearson's $r=0.28; p=0.011$), and improved sleep quality (Pearson's $r=0.37; p<0.001$).

Moreover, interest in sexual activity was reported as increased by 44 of 80 subjects (55%) [95%CI=43.5%, 66.2%] and this improvement after ovarian PRP was strongly correlated with better skin tone, nail growth, and scalp hair thickness/texture improvements (Pearson's $r=0.54; p<0.001$), higher energy level (Pearson's $r=0.37; p<0.001$), clearer thinking/improved mentation (Pearson's $r=0.39; p<0.001$), as well as better sleep quality (Pearson's $r=0.46; p<0.001$). A related change in arousal/sexual desire was also noted among study patients following ovarian PRP, such that 37 of 80 women (46.3%; 95%CI=35%, 57.8%) indicated that this was enhanced after treatment, as was the ability to achieve orgasm/climax during sex (45% reported improvement; 95%CI=33.9%, 56.5%). As shown in Figure 2, respondents answering affirmatively regarding improved level of overall sexual experience after ovarian PRP were significantly older than patients who

![Graph showing distribution of patient age as a function of treatment enrollment date among women (n=80) who completed transvaginal injection of intraovarian PRP.](image1)

**Fig. 1.** Distribution of patient age as a function of treatment enrollment date among women (n=80) who completed transvaginal injection of intraovarian PRP.

![Box plot showing improvement in overall sexual experience after ovarian PRP.](image2)

**Fig. 2.** Improvement of 'overall sexual experience' following intraovarian injection of autologous platelet rich plasma, as measured from anonymous, confidential self reports (n=80). In this sample, mean±SD age for patients responding yes vs. no were 47.9±6.3 vs. 43.5±5.2yrs (p=0.001, by two tailed t-test).
did not report such change (47.9±6.3 vs. 43.5±5.2yrs; 
p=0.001). Changes in pre- vs. post-PRP responses 
across all study parameters are summarized in Figure 3.

Although subjects who enrolled in the RCT usually 
had both ovaries treated with autologous PRP, this was 
not possible for 35% of cases (n=28). Unilateral ovar-
ian injection was generally due to limited visibility of 
adnexal structures via transvaginal ultrasound, second-
ary to body habitus. Our analysis confirmed that access 
to one ovary only was significantly correlated to BMI 
(p=0.027), such that heavier patients were less likely to 
undergo bilateral ovarian PRP treatment. Nevertheless, 
sub-analysis of survey data revealed that injection of 
autologous PRP into just one ovary was similarly effec-
tive in manifesting change in overall sex life satisfac-
tion change (p=0.85), energy level (p=0.42), and menses 
pattern change (p=0.15) compared to bilateral ovarian 
treatment.

DISCUSSION

Eventual cessation of menses heralds the natural clos-
ing of the reproductive window for females (Sills et al., 
2009), and the effects of this narrowing therapeutic 
spectrum in clinical fertility practice often include low 
ovarian reserve and menstrual irregularity. As a bio-
logical process, perimenopause and menopause may be 
regarded as universal among women of sufficient age, 
yet the constellation of symptoms can impact produc-
tivity and quality of life with much variation (Greening, 
2017). Indeed some infertility patients may not con-
front menopause until many years later, but neverthe-
less experience features of ovarian ageing—where the 
challenge of reproductive loss is but one component. 
Accordingly, fertility issues tend to take the spotlight 
during IVF consultations while not far from center-
stage are equally distressing issues including vaginal 
discomfort and dryness (Kingsberg & Krychman, 2013; 
Naumova & Castelo-Branco, 2018), reduced libido 
(Shifren et al., 2008; Cappelletti & Wallen, 2016), poor 
sleep quality (Baker et al., 2018; Jones et al., 2018), and 
cognitive decline (Berent-Spillson et al., 2013; Georgi-
kis et al., 2016).

Against this background, two publications have 
discussed ovarian tissue treatment with autologous 
platelet rich plasma (PRP) specifically as a precursor 
to in vitro fertilization. The initial paper described 
four poor-prognosis IVF patients (mean age 42yrs) 
who were consigned to donor oocyte treatment; all 
produced blastocysts for cryopreservation after ovar-
ian PRP (Sills et al., 2018) and one has since under-
gone transfer and healthy term delivery. Six months 
later, researchers in Greece reported on three poor-
responder IVF patients (mean age 38yrs) with com-
parable “revolutionary” outcomes (Sfakianoudis et al., 
2018). In the current study, low reserve with irregular 
or absent menses at baseline was corrected after ovar-
ian PRP in a significant proportion of patients, such
that more than half resumed menses and were able to discontinue exogenous hormone replacement therapy (HRT). While the observed changes may or may not be directly connected to follicular recruitment or IVF, we believe they are nevertheless important and deserve closer study.

Prior IVF data on ovarian PRP suggested that leaner women were more likely to respond to this intervention than those with higher BMI (Sills et al., 2018), and the present investigation extends this observation by noting patients lost weight and significantly reduced their BMI (p = 0.028) after ovarian PRP. Curiously, while higher BMI was linked to reduced ability to access both ovaries safely for PRP dosing, unilateral ovarian injection did not meaningfully diminish any measured qualitative outcome.

While estradiol and testosterone are both ovarian products important in modulating female sexual and neurobehavioral response (Dhanuka & Simon, 2015; Cappelletti & Wallen, 2016; Fantasia, 2016), how these sex steroids might be affected by injection of PRP into the ovary is not known. Of note, significant increases in sexual activity, improved ability to reach orgasm/climax, and better overall sexual experience were reported following ovarian PRP injection here. It is plausible that a higher level of ovarian endocrine output results from autologous PRP “rescue”, explaining why our patients reported significant improvements in sleep quality, energy level, dermatological characteristics like nails/skin/scalp hair, clarity of thinking, as well as cervical mucus production and vaginal lubrication. The finding of improved overall sexual experience among patients at significantly higher age (vs. non-responders) invites additional study and suggests ovaries in such older women could be more sensitive to or better suited for ovarian PRP.

Based on the scope of changes experienced by these patients, should ovarian PRP be considered for symptomatic women not necessarily aspiring to retrieve their own eggs? A growing body of literature now addresses ovarian senescence, usually with emphasis on lifestyle modification, calorie restriction, toxin avoidance, and especially pharmacologic interventions like assorted HRT regimes. Because symptoms can sometimes be severe and refractory, multiple strategies are often deployed simultaneously with varying efficacy. If our results can be validated by additional multicenter studies, ovarian treatment with autologous PRP could join these interventions and become a useful therapeutic addition—not just as an antecedent to IVF as initially proposed (Sfakianoudis et al., 2018; Sills et al., 2018) but for general management of systemic perimenopausal symptoms.

How might the dramatic changes observed here be explained? What is it about injecting autologous PRP into ovarian tissue—considered impaired or dormant in most cases—that could yield an apparent alteration in function? Discussion of IVF cycle data after ovarian PRP permitted some conjecture (Sills et al., 2018), and the findings reported here appear to point in the same direction. Specifically, administration of activated PRP delivers growth factors, chemokines, and cytokines such as stromal cell derived factor-1 and hepatocyte growth factor deep into ovarian tissue. Upon arrival these and other molecular signals orchestrate tissue perfusion and angiogenesis (Szafarowska & Jerzak, 2013), possibly setting the stage for ovarian re-potentiation. Indeed, placement of these PRP-derived cell signals might “switch on” adult ovaries with low or absent reserve by establishing communication channels with uncommitted ovarian stem cells, thus creating local signaling contexts to induce differentiation towards (hormonally) active follicles. As proposed earlier (Sfakianoudis et al., 2018; Sills et al., 2018), this sequence could also entail postnatal oogenesis—a pathbreaking but unsettled principle for fertility practice where research both in support (Virant-Klun et al., 2012; Woods et al., 2013) and in opposition (Byskov et al., 2011; Zhang et al., 2012) exists.

Several limitations of our work should be acknowledged. Any questionnaire used to collect post hoc data could be subject to recall bias among respondents. Here the interval between PRP intervention and clinical assessment was limited, and this method has been successfully applied to assess sexual response after other gynecology procedures (Saini et al., 2002). In addition, it would have been ideal to have captured more detail on quality of life changes over time, especially about which changes were experienced in what sequence (and duration of their effects), although this awaits further longitudinal study in larger populations. Finally, our analysis would have been substantially strengthened if the qualitative changes reported privately here were linked on a case-by-case basis to laboratory data collected after ovarian PRP. These data do exist and form the basis of further investigations, but to preserve patient confidentiality our anonymous survey could not make that connection.

In summary, while ovarian injection with autologous PRP has achieved significantly improved reserve markers (Sills et al., 2018) and yielded livebirths from poor prognosis IVF patients using their own oocytes (Sfakianoudis et al., 2018), the neurobehavioral and metabolic changes measured here position ovarian PRP beyond conventional fertility practice. Here, we offer evidence of improvement in multiple quality of life parameters following use of intraovarian PRP. To clarify which patient characteristics may predict responsiveness to ovarian PRP, as well as how best to refine this minimally invasive technique, additional clinical research is underway.

**CONFLICT OF INTEREST DISCLOSURE**

ESS holds a provisional U.S. patent for process & treatment using ovarian platelet rich plasma.
REFERENCES


Sills et al: Qualitative changes after ovarian PRP