Neuroendocrinology Letters Volume 39 No. 6 2018
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

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

E. Scott Sills [1,2,4], Xiang Li [1,3], Natalie S. Rickers [1], Samuel H. Wood [4], Gianpiero D. Palermo [5]

1 Office for Reproductive Research, Center for Advanced Genetics; La Jolla, California USA
2 Applied Biotechnology Research Group, University of Westminster; London UK
3 Paralian Technology Inc., Mission Viejo, California USA
4 Gen 5 Fertility; La Jolla, California USA
5 Center for Reproductive Medicine & Infertility, Weill Medical College of Cornell University; New York NY USA

Correspondence to: Office for Reproductive Research, CAG at Gen 5 Fertility; 4150 Regents Park Row, Suite 300, La Jolla, California 92037 USA
FAX: +1 858-225-3535; E-MAIL: drsills@CAGivf.com

Submitted: 2018-10-20 Accepted: 2018-11-02 Published online: 2018-11-28

Key words: **reproductive ageing; platelet rich plasma; ovary; sexual health; metabolism; rejuvenation**

Neuroendocrinol Lett 2018; **39**(6):427–433 **PMID:** 30796792 NEL390618A02

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/m^2, 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; Sills *et al.*, 2018). But independent of oocyte dynamics and IVF, what about broader, systemic responses following ovarian PRP? 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 pretested 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/m^2, 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 χ^2 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).

Tab. 1. Summary of items assessed by anonymous questionnaire among women who completed intraovarian injection of autologous platelet rich plasma (PRP).

1.	In what year were you born? (enter 4-digit birth year; for example, 1976)
2.	When did you receive PRP at Dr. Sills office?
3.	What is your height in feet and inches?
4.	At the time of your ovarian PRP procedure, what was your approximate weight in pounds?
5.	What is your approximate weight now, in pounds?
6.	Prior to your ovarian PRP treatment, were you having regular (approximately monthly) menses?
7.	Are you having regular (approximately monthly) periods after ovarian PRP, or has your menstrual pattern become more frequent?
8.	Prior to your ovarian PRP treatment, did you ever take prescription HRT (hormone replacement therapy)? Note - this includes birth control pills.
9.	After your ovarian PRP treatment, have you used any prescription HRT (hormone replacement therapy)? Note - this includes birth control pills.
10.	At the time of your ovarian PRP procedure, were you in a relationship which included regular sexual activity?
11.	After ovarian PRP treatment, did you continue your existing intimate relationship OR initiate a new intimate relationship?
12.	Before your ovarian PRP treatment, how would you score your overall energy/activity level?
13.	After your ovarian PRP treatment, how would you score your overall energy/activity level?
14.	Before your ovarian PRP treatment, how would you describe your personal satisfaction with skin, nails, and hair characteristics?
15.	After your ovarian PRP treatment, how would you describe your personal satisfaction with skin, nails, and hair characteristics?
16.	Before your ovarian PRP treatment, how would you score your ability to think clearly (i.e., level of mental/cognitive function)?
17.	After your ovarian PRP treatment, how would you score your ability to think clearly (i.e., level of mental/cognitive function)?
18.	Prior to ovarian PRP treatment, how would you score your ability to get a good night's sleep (i.e., sleep duration & quality)?
19.	Following ovarian PRP treatment, how would you score your ability to get a good night's sleep (i.e., sleep duration & quality)?
	The remaining questions are based on the Female Sexual Function Index (FSFI). In answering these standard queries, the following definitions apply: Sexual activity can include caressing, foreplay, masturbation, and vaginal intercourse. Sexual intercourse is defined as penile penetration (entry) of the vagina. Sexual stimulation includes situations like foreplay, masturbation, or sexual fantasy. Where: 1=almost never, 2=less than half the time, 3=about half the time, 4=more than half the time, 5=almost always.
20.	Before your ovarian PRP procedure, how often did you feel sexual desire or interest?
21.	After your ovarian PRP procedure, how often did you feel sexual desire or interest?
22.	Before your ovarian PRP procedure, how often would you note cervical mucus production or (natural) vaginal lubrication during sexual activity or intercourse?
23.	After your ovarian PRP procedure, how often would you note cervical mucus or (natural) vaginal lubrication during sexual activity or intercourse?
24.	Before your ovarian PRP procedure, how often were you satisfied with your arousal (excitement) during sexual activity or intercourse?
25.	After your ovarian PRP procedure, how often were you satisfied with your arousal (excitement) during sexual activity or intercourse?
26.	Before your ovarian PRP procedure, how often did you reach orgasm (climax) during sexual stimulation?
27.	After your ovarian PRP procedure, how often did you reach orgasm (climax) during sexual stimulation?
28.	Before your ovarian PRP procedure, how often were you satisfied with your overall sexual life?
29.	After your ovarian PRP procedure, how often were you satisfied with your overall sexual life?
30.	Were both of your ovaries able to be accessed and injected with PRP by Dr. Sills (even if this required more than one visit)?

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-

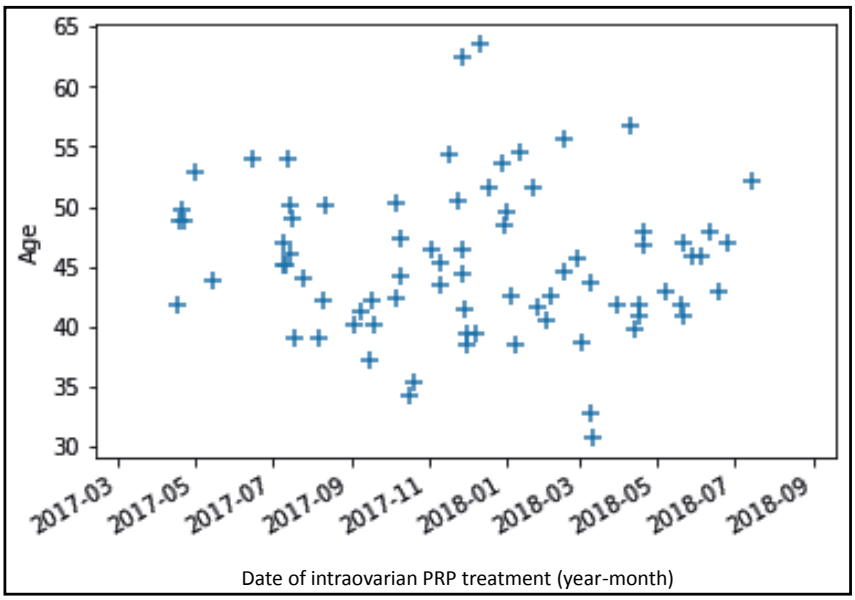

Fig. 1. Distribution of patient age as a function of treatment enrollment date among women (n=80) who completed transvaginal injection of intraovarian PRP.

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

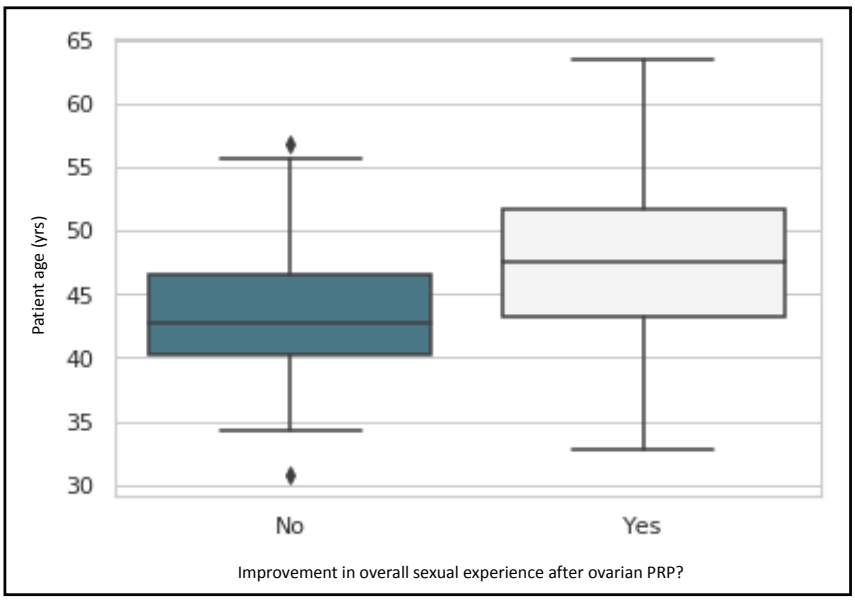

Fig. 2. Improvement of 'overall sexual experience' following intraovarian injection of autologous platelet rich plasma, as measured from anonymous, confidential self reports (n=80). In this sample, mean±SD age for patients responding yes vs. no were 47.9±6.3 vs. 43.5±5.2yrs (p=0.001, by two tailed t-test).

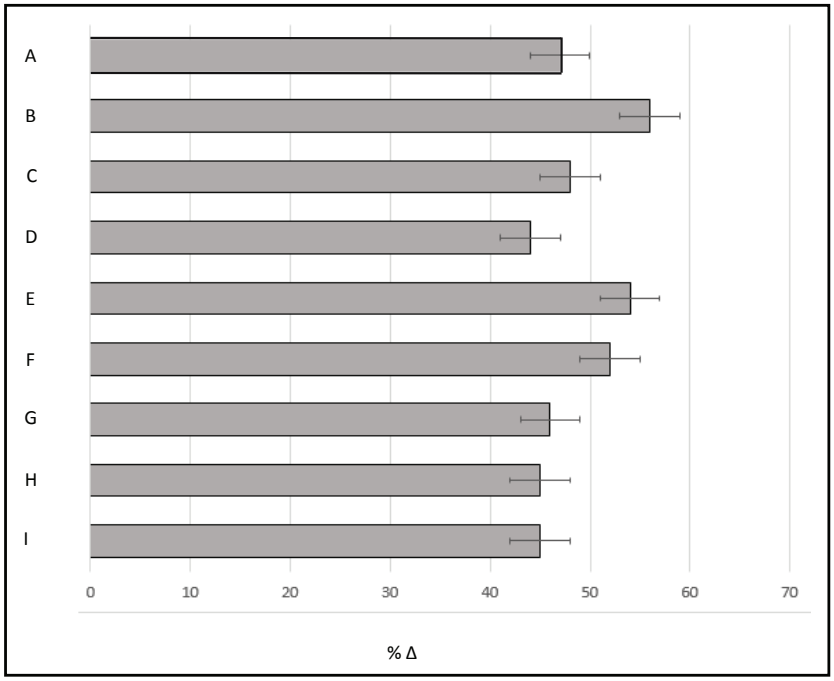

Fig. 3. Change (%Δ) in selected sexual function and metabolic parameters before vs. after intraovarian injection of autologous platelet rich plasma, as measured from anonymous, confidential self reports (n=80). Where: A=skin tone/hair thickness/ nail growth, B=daily energy level, C=cognitive clarity, D=sleep quality, E=sexual desire/libido, F=cervical mucus/vaginal lubrication, G=arousal, H=ability to achieve orgasm/climax, I=overall sexual experience.

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 ovarian injection was generally due to limited visibility of adnexal structures via transvaginal ultrasound, secondary 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 effective in manifesting change in overall sex life satisfaction 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 closing 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 biological process, perimenopause and menopause may be regarded as universal among women of sufficient age, yet the constellation of symptoms can impact productivity and quality of life with much variation (Greening, 2017). Indeed some infertility patients may not confront menopause until many years later, but nevertheless 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 centerstage 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; Georgiakis *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 ovarian PRP (Sills *et al.*, 2018) and one has since undergone transfer and healthy term delivery. Six months later, researchers in Greece reported on three poor-responder IVF patients (mean age 38yrs) with comparable "revolutionary" outcomes (Sfakianoudis *et al.*, 2018). In the current study, low reserve with irregular or absent menses at baseline was corrected after ovarian 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

1 Baker FC, Lampio L, Saaresranta T et al. (2018). Sleep and Sleep Disorders in the Menopausal Transition. Sleep Med Clin **13**(3): 443-56.
2 Berent-Spillson A, Briceno E, Pinsky A et al. (2015). Distinct cognitive effects of estrogen and progesterone in menopausal women. Psychoneuroendocrinology **59**:25-36.
3 Byskov AG, Hoyer PE, Andersen CY et al. (2011). No evidence for the presence of oogonia in the human ovary after their final clearance during the first two years of life. Hum Reprod **26**:2129–39.
4 Cappelletti M, Wallen K (2016). Increasing women's sexual desire: The comparative effectiveness of estrogens and androgens. Horm Behav **78**: 178-93.
5 Crisp CC, Fellner AN, Pauls RN (2015). Validation of the Female Sexual Function Index (FSFI) for web-based administration. Int Urogynecol J. **26**(2): 219-22.
6 Dhanuka I, Simon JA (2015). Flibanserin for the treatment of hypoactive sexual desire disorder in premenopausal women. Expert Opin Pharmacother. **16**(16): 2523-9.
7 Fantasia HC (2016). Flibanserin and Female Sexual Desire. Nurs Womens Health. **20**(3): 309-14.
8 Frigge M, Hoaglin DC, Iglewicz B (1989). Some implementations of the boxplot. Am Stat **43**: 50-4.
9 Georgakis MK, Kalogirou EI, Diamantaras AA et al. (2016). Age at menopause and duration of reproductive period in association with dementia and cognitive function: A systematic review and meta-analysis. Psychoneuroendocrinology **73**: 224-43.
10 Greening J (2017). Menopause transition: effects on women's economic participation. HM Government Equalities Office [United Kingdom]; July 20, 2017: https://www.gov.uk/government/publications/menopause-transition-effects-on-womens-economic-participation [site accessed August 20, 2018].
11 Gurtner GC, Werner S, Barrandon Y et al. (2008). Wound repair and regeneration. Nature. **453**: 314–21.
12 Jones HJ, Zak R, Lee KA (2018). Sleep Disturbances in Midlife Women at the Cusp of the Menopausal Transition. J Clin Sleep Med **14**(7): 1127-33.
13 Kingsberg SA, Krychman ML (2013). Resistance and barriers to local estrogen therapy in women with atrophic vaginitis. J Sex Med **10**(6): 1567-74.
14 Lacci KM, Dardik A (2010). Platelet-rich plasma: support for its use in wound healing. Yale J Biol Med **83**: 1–9.
15 Naumova I, Castelo-Branco C (2018). Current treatment options for postmenopausal vaginal atrophy. Int J Womens Health **10**: 387-395.
16 Nurden AT (2011). Platelets, inflammation and tissue regeneration. Thromb Haemost **105**(Suppl.1): S13–S33.
17 Saini J, Kuczynski E, Gretz HF 3rd et al. (2002). Supracervical hysterectomy versus total abdominal hysterectomy: perceived effects on sexual function. BMC Womens Health **2**(1): 1.
18 Sfakianoudis K, Simopoulou M, Nitsos N et al. (2018). A Case Series on Platelet-Rich Plasma Revolutionary Management of Poor Responder Patients. Gynecol Obstet Invest 2018: 1-8 doi: 10.1159/000491697.
19 Shifren JL, Monz BU, Russo PA et al. (2008). Sexual problems and distress in United States women: Prevalence and correlates. Obstet Gynecol **112**(5): 970–8.
20 Sills ES, Alper MM, Walsh AP (2009). Ovarian reserve screening in infertility: practical applications and theoretical directions for research. Eur J Obstet Gynecol Reprod Biol **146**(1): 30-6.
21 Sills ES, Rickers NS, Li X et al. (2018). First data on in vitro fertilization and blastocyst formation after intraovarian injection of calcium gluconate-activated autologous platelet rich plasma. Gynecol Endocrinol **28**: 1-5.
22 Szafarowska M, Jerzak M (2013). Ovarian aging and infertility. Ginekol Pol **84**: 298–304.
23 U.S. National Library of Medicine (2017). Autologous platelet-rich plasma (PRP) infusions and biomarkers of ovarian rejuvenation and ageing mitigation (NCT03178695); March 7, 2017 https://clinicaltrials.gov/ct2/show/study/NCT03178695 [site accessed August 20, 2018].
24 Virant-Klun I, Stimpfel M, Skutella T (2012). Stem cells in adult human ovaries: from female fertility to ovarian cancer. Curr Pharm Des **18**: 283–92.
25 Woods DC, White YA, Tilly JL (2013). Purification of oogonial stem cells from adult mouse and human ovaries: an assessment of the literature and a view toward the future. Reprod Sci **20**: 7–15.
26 Zhang H, Zheng W, Shen Y et al. (2012). Experimental evidence showing that no mitotically active female germline progenitors exist in postnatal mouse ovaries. Proc Natl Acad Sci USA **109**: 12580–5.