Sills and Wood
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REVIEW

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Discussion of field effects after intraovarian injection of autologous platelet-rich plasma

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Abstract

Background In the pre-menopausal ovary, the oocyte does not develop in isolation. Stroma, perivascular cells, immune cells, granulosa cells and endothelium are unequivocally active, and compelling evidence are also available placing germline stem cells within this milieu. Indeed, the local cytoarchitecture network of collagen, proteoglycans, polysaccharides, and fibrous proteins jointly influence endocrine, nutrient, and osmotic fluid movement vital to eggs. After transiting basal lamina, these moieties can directly determine follicular growth and oocyte metabolism.

Main body of the abstract Over time, this support apparatus changes to dampen crucial biochemical inputs and eventually disconnects the oocyte from its own regulatory grid. Background factors extrinsic to the oocyte such as stroma and extracellular matrix thus contribute to overall reproductive fitness. Both menopause and infertility are thus distinct clinical manifestations of a common knock-down of ovarian competence. While treatments for symptomatic menopause and infertility traditionally depend on standard hormone replacement therapy or synthetic gonadotropins, autologous platelet rich plasma (PRP) has arrived as an alternative method to improve ovarian reserve.

Short conclusion Intraovarian PRP is usually considered to interact mainly with follicles or oocyte precursors, although other ovarian components also respond to platelet cytokines. Cross-discipline PRP effects measured in similar (non-reproductive) stroma and tissue matrix systems are examined here, with a view to promote greater research bandwidth for intraovarian PRP.

Keywords Platelets, Ovary, Rejuvenation, Infertility, Menopause

Background

Main text

How many distinct cell types are resident in the adult human ovary, and are connections across these sets relevant to oocyte recruitment and support? These queries received attention in a recent transcriptional analysis of ovarian cellular components which identified endothelial cells, granulosa cells, immune cells, oocytes, perivascular cells, and stroma (Wagner et al. 2020). As the study found no ovarian stem cells among the six cell populations present in the adult human ovary, it tended to endorse the traditional paradigm concerning age-limited ovarian reserve. However, the conspicuous absence of ovarian stem cells was later explained by suboptimal centrifugation parameters which would predictably fail to capture them (Bhartiya and Sharma 2020). Based on this critique, a different laboratory protocol should update or correct the ovary cell constituent roster to include germline stem cells.

The issue again drew notice when Tocci (Tocci 2022) articulated a detailed challenge to a position framed by Oktem (Oktem 2022) in a debate on sequential stimulated IVF cycles. During this exchange unrelated to 'ovarian rejuvenation', data aligning with Bhartiya and Sharma (2020) recalled that two distinct stem cell types localize

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to the adult human ovarian surface epithelium: Very small embryonic-like (VSEL) stem cells in latency, as well as mitotically active ovarian stem cells (Parte et al. 2013; White et al. 2012; Virant-Klun et al. 2013). And within this physiologic niche, somatic cells locally facilitate the transition of ovarian stem cells to competent oocytes (Tocci 2022; Bhartiya 2015; Sills and Wood 2019).

Information on ovarian substructure and function produced from various experts permits a new merger of research themes, guiding a better understanding of intraovarian PRP. For example, when embryology/IVF outcomes data after intraovarian PRP were first published (Sills et al. 2018), two mechanisms were hypothesized for the follicular action of platelet cytokines within the adult ovary. Unfortunately neglected in that proposal were any putative extra-follicular contributions evoked by PRP, discussed here to revise and extend prior work.

PRP: Juxta-follicular considerations

Descriptions of autologous platelet growth factors in women's reproductive health tend to center on PRP (or its derivatives) placed into the uterus or ovarian tissue. For the latter, the focus is generally on follicular or oocyte responses but a therapeutic contribution by PRP to targets outside the oocyte should not be discounted. The observation that intraovarian injection of fresh, autologous activated PRP increased serum anti-mullerian hormone (AMH) in poor-responder fertility patients, and that this effect was correlated to baseline platelet concentration rather than age or infertility duration (Sills et al. 2018) implicates granulosa cells as a key operator in PRP response. While prior work has outlined how recruitment of de novo oocytes, presumably from latent ovarian stem cells may explain how intraovarian PRP works (Sills and Wood 2019), how this intervention might change the extra-follicular milieu remains to be characterized.

To appreciate the structure and function of the extracellular matrix, an inventory of relevant proteins is essential (Hynes and Naba 2012). That PRP or its cytokine derivatives may impact connective tissues or ovarian stroma is inferred from actions (Cenni et al. 2009; Wang et al. 2021; Scopelliti et al. 2022; Moulavi et al. 2020; Liu et al. 2022; Ahmed et al. 2006) in better studied non-ovarian settings (see Table 1).

Discussion

When human platelet-derived cytokines as purified exosome products are cultured with various endometrial specimens, uptake of platelet factors yields marked cell proliferation and enhanced wound healing (Miller et al. 2022). This advances prior research (Sak et al. 2013) which found growth factors at midcycle to be lower in recurrent implantation failure vs. healthy controls. Autologous platelet cytokines placed into the endometrial compartment to rectify this shortage did increase embryo implantation (Nazari et al. 2016; Hajipour et al. 2021), suggesting a beneficial role at the blastocyst-endometrium connective tissue interface. Although crosstalk between ovarian follicles and the extracellular field is important, this is often eclipsed by direct receptor-ligand interactions involving membrane bound receptors at the follicle surface.

As discussed previously (Rickers and Sills 2022), a high leukocyte fraction in the platelet releasate generally favors production of proinflammatory cytokines likely impacting Type I collagen synthesis and alkaline phosphatase by osteoblasts (Baca-Gonzalez et al. 2022). Dermatology data compiled by Yunus et al. (2022) found PRP to be an effective therapeutic modality for skin rejuvenation, wound healing, and androgenic alopecia. Another cosmetic condition of unknown etiology is melasma, a superficial connective tissue pigmentation disorder where management with PRP has been closely studied (Tuknayat et al. 2021; Magistretti et al. 2022). Since PRP can produce such corrective or regenerative effects

Table 1 Ovary components by cell type and corresponding response to platelet-rich plasma or platelet (PLT) derivatives, based on reported corollary observations in each group

Classification	PLT protocol	Field effect	Refs
Endothelial	N	PDGF-B & ICAM-1 expression, cell migration	Cenni et al. 2009)
Granulosa	+ hMSC	↑ proliferation, ↓ apoptosis	Wang et al. 2021)
Immune	N	macrophage & neutrophil activation	Scopelliti et al. 2022)
Oocyte	N	↑ mitochondrial activity	Moulavi et al. 2020)
Perivascular	N	↑VEGF, ↑ CD34 expression, ↑ perfusion	Liu et al. 2022)
Stroma	EGF, MMP-2 & 9	epithelial → mesenchymal transition	Ahmed et al. 2006)
Germline stem	*Pending*	*Undefined*	–

N = neat (unfractionated), PDGF-B = platelet derived growth factor subunit B, ICAM-1 = intercellular adhesion molecule-1 (CD54), hMSC = human mesenchymal cells, VEGF = vascular endothelial growth factor, CD34 = hematopoietic progenitor cell marker, EGF = epidermal growth factor, MMP = matrix metalloproteinase

involving connective tissue outside the ovary, comparable activity within the ovary also seems plausible.

Of note, Luyckx and colleagues (2014) were the first to report survival & growth of isolated murine ovarian follicles after autologous ovarian cell transplant to a fibrin scaffold. Experimental evidence of platelet-derived factors as matrix-modulators also exists from bioengineered cartilage with cells able to migrate on PRP fibers to form cartilaginous tissue (Wu et al. 2022). Similar tissue engineering research (Zhao et al. 2022) has detailed PRP-integrated alginate gelatin composite hydrogel bioinks, where PLT cytokines appear to assist seeded cell behavior, form vascular endothelial cells, and organize macrophage polarization in a paracrine manner. When damaged cornea was treated with PRP, the basal epithelium, intermediate layer, and the superficial squamous layer all appeared histologically indistinguishable from healthy controls (Hashem 2020). If similar connective tissue effects were operant in ovarian analogs after PRP treatment, this would be distinct from oocyte programming and development to help explain early results (Sills et al. 2018). As ongoing work continues, use of PRP in other (e.g., in situ bioprinting) contexts has already shown it can contribute to angiogenesis, modulated inflammation, and faster wound closure (Zhao et al. 2022).

From serum AMH responses observed after intraovarian PRP (Sills et al. 2020), it is possible that post-treatment ovarian effects beyond the oocyte are also regulated by ambient PLT concentration. In addition to baseline PLT number, the activation particulars for PLTs offer opportunity for refinement. Cytokines and growth factors may be released via PLT activation using 100 mg/mL calcium gluconate (10%), 100 ng/mL convulxin, 50 μM TRAP-6, 5 nM thrombin, as well as other reagents (Rickers and Sills 2022; Heinzmann et al. 2021). One drawback of 'ovarian rejuvenation' may be its relatively brief biological activity for connective targets (Rao et al. 2022). However, this attribute may ultimately explain why a hyperstimulation response has never been reported with intraovarian PRP (Sills et al. 2022). Ongoing research may supply some answers once specific ovarian stromal responses following PRP are elucidated. For example, signaling changes induced in stroma after intraovarian PRP may enable or enforce moving germline stem cells into an oocyte lineage (Sills and Wood 2022).

Notwithstanding any putative restorative action on ovarian follicles/oocyte precursors, research in other areas has confirmed that platelet-derived cytokines do have effects on connective tissue (Rastegar Adib et al. 2022). This has special relevance to clinical fertility practice, because ovarian aging entails poor vascularization, oxidative stress by excessive free radicals, or advanced glycation end products in the follicular stromal neighborhood (Szafarowska and Jerzak 2013; Wood and Sills 2020; Mouanness et al. 2022). If autologous PRP can ameliorate these imbalances, the transformation for women's health would be difficult to overstate.

In summary, it is generally agreed that PLT isolation technique, as well as activation and handling methods will determine both reagent quality (Bieback et al. 2019) and subsequent ovarian response (Sills 2022). Because there is considerable variation in PLT processing techniques and injection method, this lack of standardization has limited the clinical uptake of this investigational treatment (Rickers and Sills 2022). In the meantime, proposed PRP mechanisms of action have favored placing oocyte precursors or the follicle at center stage. However, the ovarian microclimate also does help direct egg development, so background modifications here by PRP could likewise influence follicle assembly and impact oocyte competency (Fiorentino et al. 2022).

Conclusions

Since cell groups beyond the follicle respond to intraovarian PRP, research on stromal PRP effects in non-reproductive tissue is relevant. Cytokines of PLT origin likely manifest congruent action in the adult human ovary; additional study can help delineate contributions autologous PRP may make which are synergistic with (yet distinct from) the oocyte or follicle.

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ESS organized the research plan; ESS and SHW reviewed the literature and developed revisions; both authors read and approved the final manuscript.

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