Immunology in Infertility & ART: Evidence-Based Assessment and Targeted Interventions

Precision Diagnosis in RPL: Stratifying Patients by Endometrial and Peripheral Immune Biomarkers

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Introduction

Current landscape…

- ~50% of RPL cases remain ***unexplained*** even after standard evaluation
- Off-label immunotherapy is used without reproducible patient selection criteria

Why it matters…

- Unclear immune phenotyping leads to inconsistent counselling
- Treatment debates persist because patient selection has been biologically under-specified

ESHRE, 2023; AJRI, 2025

Guidelines say…

Society / Guideline	Immune Testing	Immunotherapy	Key qualifier
ASRM / ESHRE 2023	APS testing recommended; others not validated for routine use	Not recommended outside APS	*Evidence judged insufficient for most immune interventions*
ASRI 2025	Conditional support for selected immune phenotyping with specialist expertise	May be considered in **biomarker-defined** patients	*Phenotyping + specialist guidance required*

ASRM, 2012; ESHRE, 2023; AJRI, 2025

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Evolving from: Strict empiricism ➔ **Targeted**, biomarker-guided therapy
Key gap to consider: ***Standardization*** of testing protocols & ***defining*** "actionable" biomarkers

ASRM, 2012; ESHRE, 2023; AJRI, 2025

From “empiric” treatment to “precision” care

Integrated signatures

- Single markers are *rarely* enough
- Blood, EM, cell state, and clinical phenotype should be interpreted as a **composite** immune profile

Dynamic monitoring

- *Static* values ***miss*** the biology of transition
- The **immune shift** from prepregnancy to early gestation may be more informative than a single time point

“Phenotype”-matched treatment

- The question is no longer “Does immunotherapy work in all RPL?” but **“Which immune phenotype maps to which intervention?”**

Cavalcante et al., Am J Reprod Immunol 2025; ESHRE, 2023

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Total uNK cell counting

Why it initially seemed promising

- uNK cells are biologically central at the maternal–fetal interface
- Counting them once appeared to be a tractable clinical test

Where systematic review evidence now stands

- Major heterogeneity across studies in biopsy timing, staining, gating, and thresholds
- Total CD56+ count does not stably predict pregnancy outcome
- No significant difference in LBR: high vs normal uNK
  (RR 1.00; Von Woon 2022)

Could still suggest…

- Altered immune milieu; but not mechanism, not treatment candidacy on its own

Next step?

- Switching from…
- Simple counting → **phenotype**, **subset**, **cell state**

Von Woon et al., Hum Reprod Update 2022;

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Single-cell studies

- Single-cell profiling shows that RPL is characterized by immune compositional changes, not overabundance of one cell type
- Growth-supporting dNK1-like populations are reduced in RPL decidua
- Inflammatory T-cell and cytotoxic programs expand — consistent with loss of local immune tolerance

Three questions that replace total count

- Which subsets are missing?
- Which inflammatory states are expanded?
- How is trophoblast-supportive biology being lost?

Guo et al., Cell Discov. 2021; Wang et al., 2021

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Emerging cellular targets in decidua

CCR8+ decidual Tregs

- Highly suppressive and enriched in normal early pregnancy decidua
- ***Depleted*** in RPL — linking failed tolerance to pregnancy loss

Animal model evidence *(Li et al., Science Immunology 2025)*

- Adoptive transfer of CCR8+ Tregs rescued fetal loss in abortion-prone mouse model
  - a rare example of mechanistic reversibility

Why this signal is interesting

- Beyond association — a biologically coherent cellular axis with translational potential

Keep in mind…

- Animal model data only; human trial data targeting CCR8+ Treg pathways are not yet available

Li et al., Sci Immunol. 2025

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Beyond biopsy: spatial and non-nvasive approaches

Spatial transcriptomics

- Adds positional context: healthy decidua shows implantation-zone enrichment of dNK1 and dM2-like programs
- RPL shows loss of supportive niches and expansion of cytotoxic signatures

Menstrual effluent profiling

- Biopsy-free access to the uterine immune ecosystem
- Potential for serial monitoring — correlation with in situ findings still being established

Issues to be further resolve…

- Assay harmonization/timing/reference ranges/external validation
- ➢ All required before informing treatment decisions
- EM profiling is becoming deeper and less invasive simultaneously; but still, validation required…

Sha et al., GPB 2025; Vomstein et al., RBO 2023

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Sha et al., GPB 2025; Vomstein et al., RBO 2023

MINI SUMMARY

EM profiling: current evidence

- **Total uNK count**: biologically relevant, but no validated threshold or stable LBR prediction — systematic review confirms inconsistency
- The informative signal is qualitative: dNK1 ↓, cytotoxic programs ↑, CCR8+ Tregs ↓ — phenotype matters more than total number
- **Guidelines:** ESHRE/SOGC *still* do *not* recommend routine NK testing; ASRI conditionally supports it in biomarker-defined patients, **specialist** settings only
- Spatial and non-invasive tools are emerging; yet, assay standardization/reference ranges/prospective validation are still needed

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Established guidelines…

- Summary of recommendations on thyroid evaluation for RPL

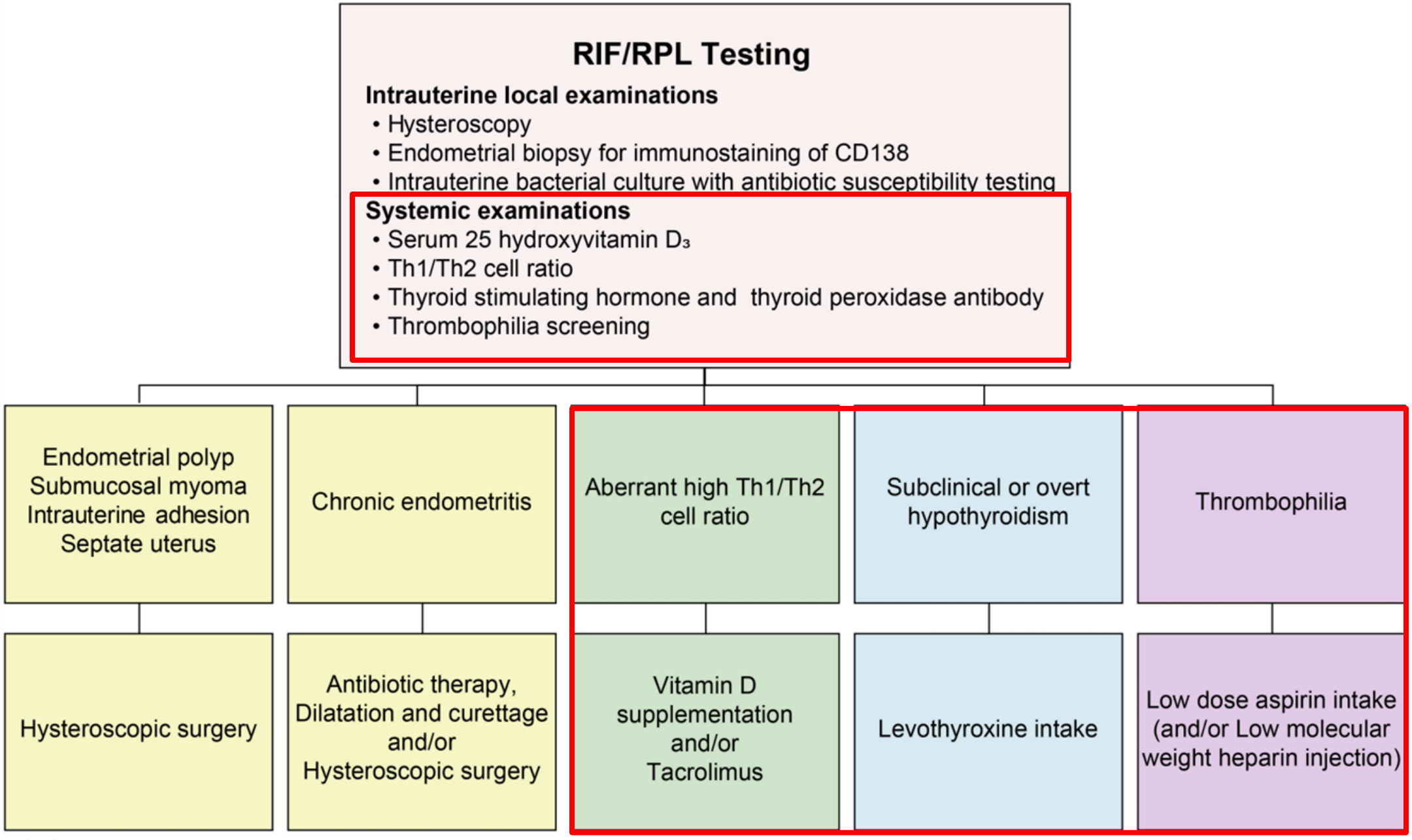
	RCOG	ESHRE	ASRM	DGGG, OEGGG and SGGG	CNGOF
Country	UK	EU	US	Germany, Austria, Switzerland	France
Issued	2011	2019/2023	2012/2020	2018	2016
Descriptions	Not recommended	**TSH** and **TPO Ab** screening is recommended	**TSH** screening is recommended	**TSH** screening is recommended	Screening for hypothyroidism (**TSH, TPO Ab**, and antitriglyceride antibodies) is recommended
		If *abnormal*, **T4** levels should be measured	If normal, T4 and thyroid antibody testing not routinely recommended	If *abnormal*, **fT3**, **fT4**, and **thyroid antibodies must** be determined	

Established guidelines…

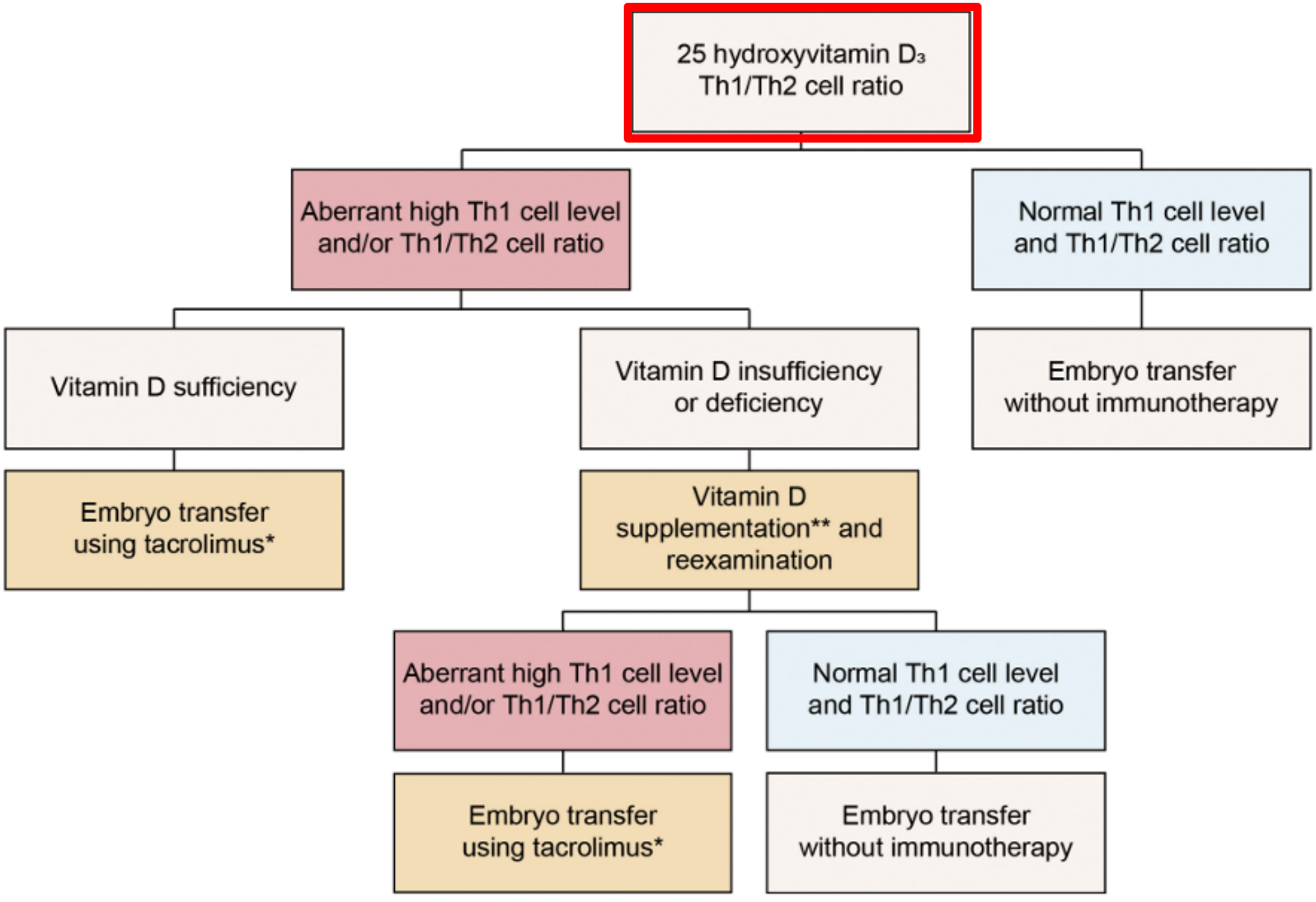
- Summary of recommendations on thrombophilia screening for RPL

	RCOG	ESHRE	ASRM	DGGG, OEGGG and SGGG	CNGOF
Country	UK	EU	US	Germany, Austria, Switzerland	France
Issued	2011	2019/2023	2012/2020	2018	2016
Descriptions	**Screening for APS recommended**				

For women with RPL, we recommend screening for antiphospholipid antibodies (LA and ACA [IgG and IgM]), after two pregnancy losses.	Strong	⊕⊕■■
For women with RPL, screening for aβ2GPI can be considered after two pregnancy losses.	GPP	



Kuroda et al., Reprod Med Biol. 2024



Kuroda et al., Reprod Med Biol. 2024

Other options

Vitamin D *(ASRI 2025)*

- 25-OHD <30 ng/mL = insufficiency
- Supplementation reduces Th1/Th2 ratio in 80-90% *(Kuroda et al., 2024)*
- Low-cost, safe, immunomodulatory; could be a practical first step

Th1/Th2 ratio (OPTIMUM framework)

- IFN-γ/IL-4 producing CD4+ T cell ratio; threshold ≥11.8 for tacrolimus tx.
- Th1/Th2↑ & Vit.D↓ → Vit.D supplementations first
  - 80-90% of patients show **ratio reduction** after vitamin D for 2 mo
  - Remaining elevated → tacrolimus (2-3 mg/day)

ASRI, 2025; Kuroda et al., Reprod Med Biol. 2024

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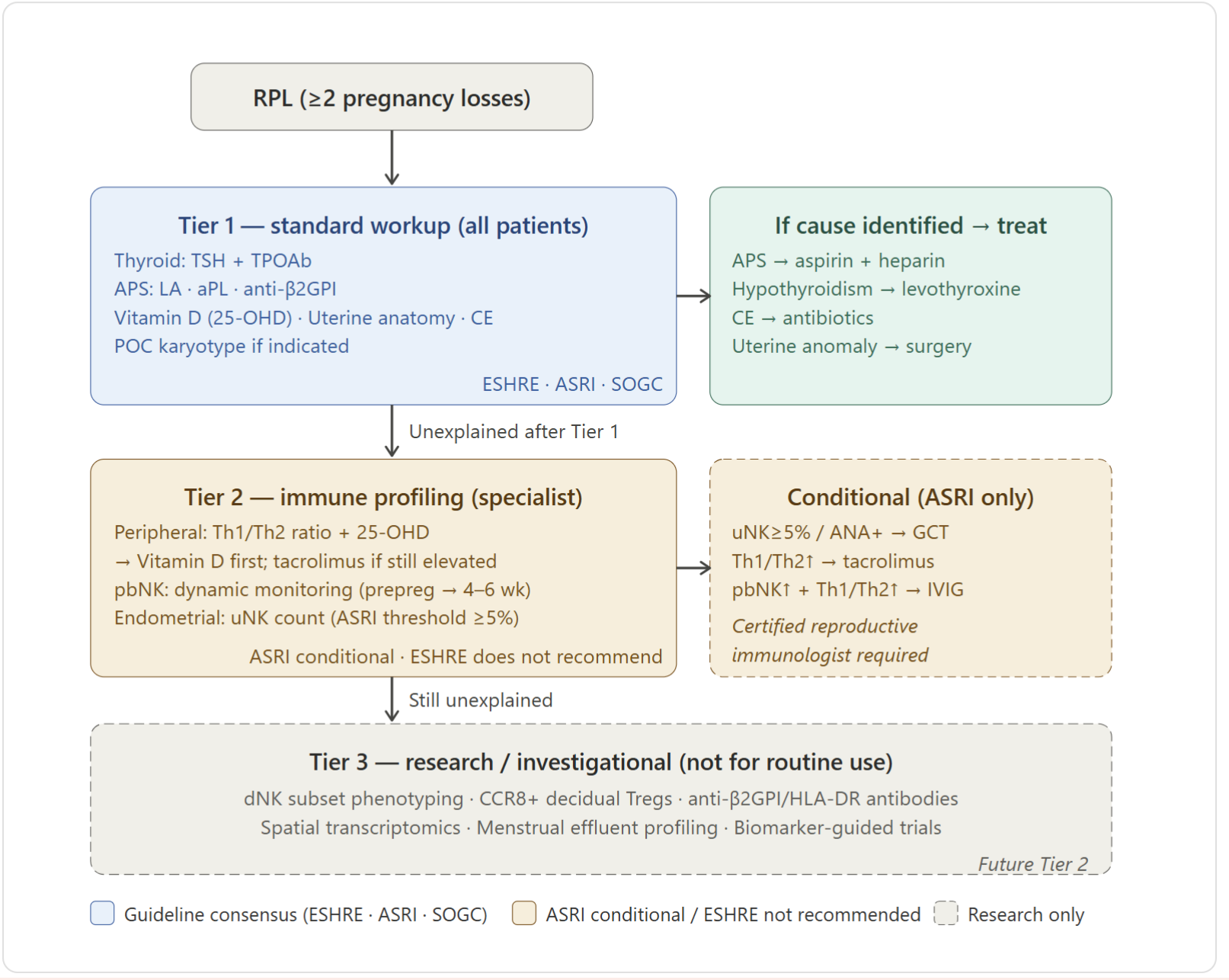
Summary

- The biology seems promising; the **biomarker** infrastructure is not yet ready
  - dNK subsets, CCR8+ Tregs, dynamic NK transition: compelling signals
  - Issues to conquer: standardization, validated thresholds, and prospective confirmation
- **Guidelines diverge** because the **evidence** is *genuinely uncertain*
  - ESHRE / SOGC vs ASRI reflects different thresholds for acting on uncertain evidence
- Stratification starts with “Tier 1”(=guidelines)
  - Thyroid/APS/uterine factors first
  - Vit. D correction: suggested to reduce Th1/Th2 imbalance in 80-90% of deficient patients
- Future perspectives
  - In clinical perspective, **standardized** assays/reference ranges and adequately powered prospective trials that align biomarker, mechanism, and intervention would be preferred

Stratification Framework: Precision Diagnosis in RPL by Immune Biomarkers

Lee HJ · 2026 대한생식면역학회 춘계학술대회 · Proposed framework based on ESHRE 2023, ASRI 2025, SOGC 2025, Kuroda 2024, Ou et al. AJOG 2024



경청해 주셔서 감사합니다.

Questions & Comments