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Spuriously elevated estradiol due to immunoassay interference in the workup of a suspected ovarian tumor: Diagnostic pitfalls and laboratory workflow recommendations

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ABSTRACT

Background: Falsely elevated estradiol levels due to analytical interference are rare but clinically relevant diagnostic pitfalls, particularly when results suggest an estrogen-producing tumor.
Case presentation: We report the case of a 51-year-old woman with markedly increased estradiol concentrations inconsistent with her clinical presentation and hormonal status. Persistently elevated estradiol values, together with imaging findings and concern for granulosa cell tumor, contributed to the diagnostic workup of a suspected estrogen-producing ovarian tumor. Bilateral salpingo-oophorectomy was performed within a multifactorial preoperative assessment; histopathology showed no evidence of malignancy.
Laboratory investigation: Postoperatively, estradiol remained markedly elevated despite gonadotropin values consistent with surgical menopause. *Re*-measurement on an alternative immunoassay platform revealed low postmenopausal estradiol levels, whereas the original competitive chemiluminescent assay continued to show persistently elevated values. Polyethylene glycol precipitation produced a substantial reduction in estradiol concentration, supporting immunoglobulin-mediated analytical interference, most likely due to heterophile antibodies.
Discussion: This case highlights the vulnerability of competitive immunoassays to analytical interference, which may paradoxically generate falsely elevated results through signal suppression. Clinical–biochemical discordance should prompt timely communication between clinicians and laboratory specialists, repeat testing, alternative-platform measurement, and targeted interference studies.
Conclusion: Recognition of analytical interference is essential to prevent misdiagnosis and avoid unnecessary or potentially harmful interventions. We propose a practical diagnostic workflow to support the identification and management of discordant estradiol results in routine laboratory practice.

1. Introduction

Estradiol is a critical biomarker in evaluating female reproductive and endocrine status [1]. Accurate measurement is essential for clinical decisions, especially when elevated levels raise suspicion for estrogen-producing tumors [2–5].

Immunoassays are widely used in clinical endocrinology due to their simplicity, low cost, and suitability for high-throughput testing [6]. Two main formats are commonly applied: immunometric (sandwich) assays and competitive assays. In immunometric assays, the analyte is captured between two antibodies: one immobilized on a solid phase and the other labeled for detection. These antibodies bind distinct epitopes on the analyte, forming a "sandwich" complex that allows for quantification. This format is suitable for larger molecules with multiple binding sites [6]. However, for small molecules like estradiol, which typically lack two distinct epitopes, competitive immunoassays are preferred. In this

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approach, a fixed amount of labeled analyte competes with the patient's endogenous analyte for binding to a limited number of antibody sites on the solid phase. The signal generated by the label is inversely proportional to the concentration of the analyte in the patient's sample, unlike immunometric assays where the signal increases with analyte concentration [7]. These assays frequently inform diagnostic and therapeutic decisions [8].

However, immunoassay-based estradiol tests can be affected by interference from heterophile or anti-animal antibodies, leading to false results and potentially unnecessary interventions [9–11].

Common interfering agents include heterophile antibodies, therapeutic monoclonal antibodies, autoantibodies, rheumatoid factor, and various cross-reacting molecules that compete with the analyte for antibody binding sites. Among competitive immunoassays, cross-reacting compounds represent the most frequent cause of analytical interference [12–14].

Falsely elevated estradiol levels due to analytical interference are rare, and are most frequently attributed to cross-reacting substances, such as the selective estrogen receptor degrader fulvestrant [15] or the aromatase inhibitor exemestane [16].

1.1. Case report

A 51-year-old woman was referred to our endocrinology unit after a routine blood test revealed extremely high estradiol levels (>8000 pg/mL; >29,360 pmol/L). Remarkably, she exhibited no clinical signs of hyperestrogenism. Physical examination was unremarkable, and the patient denied any hormonal treatment or substance use. Nevertheless, occupational history revealed the patient's exposure to various chemical agents potentially associated with her professional activity as a hairdresser. Additionally, the patient reported living in a household environment with domestic animals.

Due to the long-lasting abnormal estradiol values, different examinations had been previously performed by a gynecologist. Ultrasound evaluation revealed in her right ovary a solid lesion with cystic components, showing mild increased metabolic activity at fluorine-18-fluorodeoxyglucose positron emission tomography/computed tomography ([17] F-FDG PET/CT). An ovarian granulosa cell tumor was suspected, and bilateral salpingo-oophorectomy was performed; histopathological examination of the resected specimen showed a follicular cyst and multiple corpora albicantia, with no evidence of granulosa cell tumor or malignancy.

The decision to proceed with bilateral salpingo-oophorectomy was made in the context of a multifactorial preoperative assessment, including persistent markedly elevated estradiol concentrations, imaging evidence of a right ovarian lesion with cystic components, mild increased metabolic activity on FDG PET/CT, and clinical suspicion of granulosa cell tumor.

Timeline and milestones related to diagnosis and intervention are shown in Fig. 1.

Post-surgery, serum estradiol remained similarly elevated. At this stage, the laboratory was consulted because the persistence of markedly elevated estradiol concentrations was discordant with the postoperative gonadotropin profile and the patient's clinical status. Estradiol was therefore re-measured using an alternative immunoassay platform.

Postoperative gonadotropin values were consistent with surgical menopause, whereas estradiol remained markedly elevated, indicating clinical–biochemical discordance. Hormone levels are shown in Table 1.

These results suggested a discordance between estradiol levels and physiological status.

Estradiol was re-measured using two different immunoassay platforms: Atellica® (Siemens), which showed persistently elevated levels, and Alinity® (Abbott), which revealed low postmenopausal values (<24 pg/mL; <88 pmol/L). Because assay interference was suspected, polyethylene glycol (PEG) precipitation was performed by mixing patient serum with PEG solution according to the laboratory protocol for immunoglobulin precipitation. A 30% PEG 6000 solution (Sigma-Aldrich, Zwijndrecht, the Netherlands) was prepared in 0.1 0.1 mol/L phosphate buffer (pH 7.4). Samples were treated to achieve a final PEG 6000 concentration of 3% and incubated at 4 °C. Immune complexes were subsequently removed by centrifugation at 700 ×*g* for 45 min at 4 °C. After centrifugation, estradiol was measured in the supernatant.

Following PEG precipitation, estradiol measured on the Atellica platform decreased from 8195 pg/mL (30,080 pmol/L) to 2000 pg/mL (7340 pmol/L), corresponding to an approximately 76% reduction. Although the post-PEG value remained above the expected postmenopausal range, the marked reduction after immunoglobulin precipitation supported the presence of immunoglobulin-mediated analytical interference. The disagreement between estradiol values obtained using different methods is shown in Table 2.

Reviewing the immunoassay components, we noted that Atellica® uses sheep-derived monoclonal antibodies, while Alinity® uses rabbit polyclonal antibodies. The patient may have developed anti-sheep antibodies, potentially from environmental or occupational exposure,

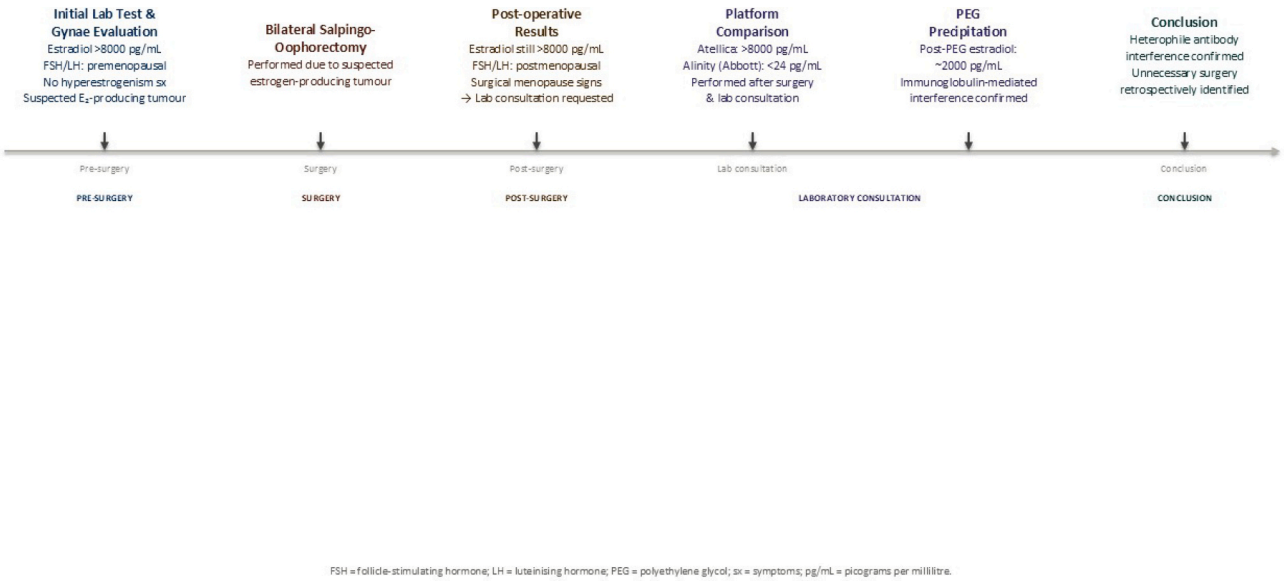

Fig. 1. Timeline of clinical events and laboratory investigations.

Table 1
Hormonal profile before and after bilateral salpingo-oophorectomy.

Hormone	Pre-surgery value	Post-surgery value	Reference interval
Estradiol, pg/mL (pmol/L)	12,383 (45,463)	8195 (30,080)	Postmenopausal: <24 pg/mL (<88 pmol/L)
FSH, IU/L	5.48	99.3	Postmenopausal: 23.0–116.3 IU/L
LH, IU/L	3.6	50.5	Postmenopausal: 5.0–55.2 IU/L
Progesterone, ng/mL (nmol/L)	35.2 (111.9)	0.26 (0.83)	Postmenopausal: not detected–0.73 ng/mL (not detected–2.32 nmol/L)

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone. SI equivalents are provided in parentheses.

Table 2
Estradiol measurements by platform before and after PEG precipitation.

Platform	Pre-PEG estradiol, pg/mL (pmol/L)	Post-PEG estradiol, pg/mL (pmol/L)	Percentage reduction	Clinical interpretation
Siemens Atellica®	8195 (30,080)	2000 (7340)	76%	Persistent elevation with marked post-PEG decrease; supports immunoglobulin-mediated interference
Abbott Alinity®	<24 (<88)	Not performed	Not applicable	Consistent with expected postmenopausal estradiol concentration

Abbreviations: PEG, polyethylene glycol. SI equivalents are provided in parentheses.

interfering with the Atellica® assay. As summarized in Table 3, differences in antibody source and assay design may lead to platform-specific susceptibility to interference, highlighting the importance of method comparison in cases of clinical–biochemical discordance.

Of note, prior investigations revealed normal values for rheumatoid factor, serum immunoglobulin levels, and protein electrophoresis.

Additional tumor markers, including CA-125, were evaluated during the preoperative workup and were within the reference range (CA-125: 18 U/mL (18 kU/L); reference interval: <35 U/mL (<35 kU/L), Siemens Atellica IM CA 125II assay).

2. Discussion

We report the case of a 51-year-old woman with markedly elevated serum estradiol concentrations that were inconsistent with her clinical presentation and hormonal profile. Estradiol measurement is a key component of endocrine and gynecological evaluation, particularly when estrogen-producing tumors are suspected [1–3]. During the preoperative evaluation, persistently high estradiol values, together with imaging evidence of a right ovarian lesion with cystic components and mild FDG uptake, contributed to the suspicion of an estrogen-producing ovarian tumor. Bilateral salpingo-oophorectomy was therefore performed within a multifactorial clinical assessment. Histopathology showed a follicular cyst and multiple corpora albicantia, with no evidence of granulosa cell tumor or malignancy.

The persistence of markedly elevated estradiol after surgery, despite postoperative gonadotropin values consistent with surgical menopause and symptoms of hypoestrogenism, represented a clear clinical–biochemical discordance. This prompted laboratory investigation. *Re*-measurement on an alternative immunoassay platform showed postmenopausal estradiol concentrations, whereas the original platform continued to report markedly elevated values. In addition, PEG precipitation produced a substantial reduction in estradiol measured on the original platform, supporting immunoglobulin-mediated analytical interference.

Table 3
Practical interpretation of assay-specific susceptibility to interference.

Feature	Atellica (Siemens)	Alinity (Abbott)	Practical implication
Antibody source	Sheep-derived antibodies	Rabbit-derived antibodies	Different animal sources may lead to assay-specific interference
Susceptibility to heterophile antibodies	Higher	Lower	Consider alternative platforms when results are inconsistent
Assay agreement	Discordant results observed	Results consistent with clinical status	Discordance suggests analytical interference rather than true pathology

Heterophile antibodies and human anti-animal antibodies may interfere with immunoassays by interacting with assay antibodies or other reagent components [6,18–22]. Although such interference is classically associated with sandwich immunoassays, competitive assays may also be affected [6]. In competitive immunoassays, signal suppression may paradoxically generate falsely elevated analyte concentrations [6]. In the present case, the discrepancy between Siemens Atellica® and Abbott Alinity® results suggests platform-specific interference, possibly related to differences in antibody source, assay architecture, or detection chemistry.

False elevations of estradiol due to analytical interference are uncommon but clinically relevant. Reported causes include cross-reactivity with drugs structurally related to estradiol, such as fulvestrant and exemestane [15], [16] biotin interference [23], and heterophile antibody-mediated effects [9,13,24–26]. The present case adds to the limited literature by documenting a strongly suspected heterophile antibody-mediated interference affecting a competitive chemiluminescent estradiol immunoassay, with relevant implications for diagnostic decision-making.

The main lesson of this case is not that estradiol immunoassay interference alone caused surgery, but that unrecognized analytical interference may reinforce a complex diagnostic pathway when laboratory results are not promptly reconciled with the clinical picture. Early communication between clinicians and laboratory specialists is therefore essential when hormone results are physiologically implausible, discordant with gonadotropins, or inconsistent with symptoms and imaging findings [6,9–13].

This report has some limitations. Serial dilution studies and heterophile-blocking tube testing were not performed, as they were not available in the routine laboratory workflow at the time of investigation. Estradiol measurement by liquid chromatography – tandem mass spectrometry (LC-MS/MS) was also not performed because it was not available in our hospital laboratory and was not subsequently requested once the alternative platform result was consistent with the clinical and hormonal context. LC-MS/MS is generally considered the reference approach for steroid hormone measurement when immunoassay interference is suspected or when results remain clinically unresolved [8] [14],., [12] Therefore, although platform comparison and PEG precipitation strongly support immunoglobulin-mediated interference, the precise nature of the interfering antibody cannot be definitively

established.

2.1. Laboratory implications

This case supports a pragmatic laboratory approach to suspected estradiol immunoassay interference. Interference should be considered

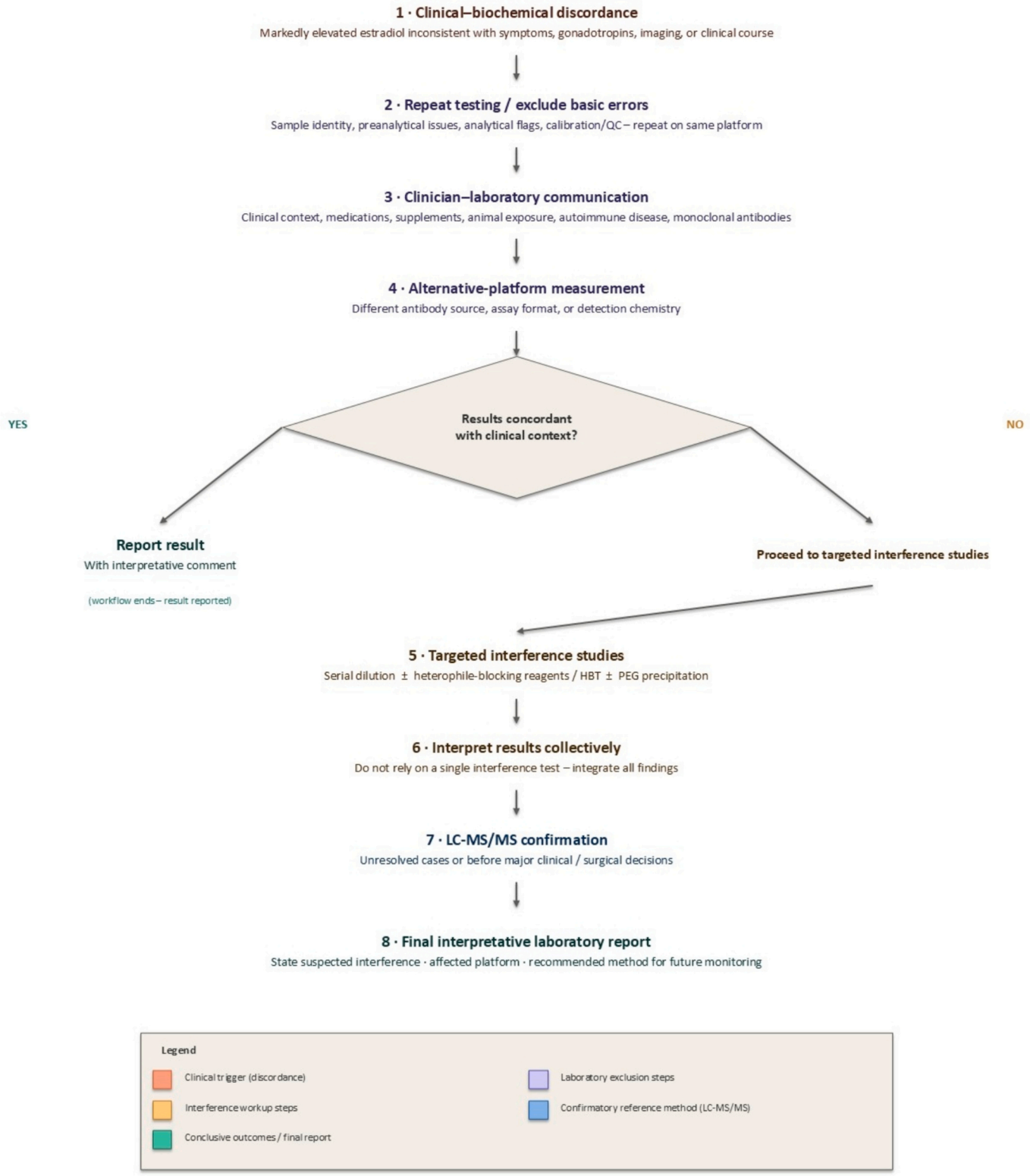

Fig. 2. Proposed diagnostic workflow for suspected estradiol immunoassay interference. The workflow emphasizes early recognition of clinical–biochemical discordance, repeat testing, clinician–laboratory communication, alternative-platform measurement, targeted interference studies, including serial dilution, heterophile-blocking reagents, and PEG precipitation when appropriate, and LC-MS/MS confirmation in unresolved or clinically critical cases.

when estradiol concentrations are markedly elevated but inconsistent with the clinical presentation, gonadotropin profile, menopausal status, imaging findings, or response to surgical or therapeutic interventions.

A stepwise approach may be useful in routine practice. First, pre-analytical or analytical errors should be excluded by repeat measurement on the same platform and review of sample integrity, calibration, and quality control data. Second, early communication between clinicians and laboratory specialists should be established to evaluate whether the result is physiologically plausible. Third, if discordance persists, estradiol should be measured using an alternative platform, preferably based on different antibody species, assay design, or detection chemistry. A marked discrepancy between platforms should raise suspicion of method-specific interference.

Targeted interference studies may then be selected according to local availability. Serial dilution studies can assess assay linearity, while heterophile-blocking reagents or heterophile-blocking tubes may help identify antibody-mediated interference. PEG precipitation may provide rapid supportive evidence for immunoglobulin-mediated interference, although it should not be interpreted as a stand-alone confirmatory test because matrix effects, incomplete removal of interfering components, and partial analyte loss may affect results.

LC-MS/MS remains the reference method for estradiol measurement and should be considered when immunoassay results remain unresolved, when discordance persists despite alternative-platform testing, or when estradiol concentrations may influence major therapeutic or surgical decisions.

A proposed diagnostic workflow for suspected estradiol immunoassay interference is shown in Fig. 2.

3. Conclusion

In the present case, spuriously elevated estradiol concentrations contributed to the diagnostic workup of a suspected estrogen-producing ovarian tumor in a clinical context that also included imaging abnormalities. The case underscores the importance of early recognition of laboratory–clinical discordance and timely communication between clinicians and laboratory specialists before major clinical decisions are undertaken.

CRediT authorship contribution statement

Gelsy Arianna Lupoli: Data curation, Conceptualization. **Carmela Polito:** Data curation. **Giuseppe Jannuzzi:** Formal analysis. **Mariano Fiorenza:** Data curation. **Ada Marino:** Data curation. **Lavinia Di Meglio:** Investigation. **Domenico Salvatore:** Supervision. **Daniela Terracciano:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Conceptualization.

Consent

Written informed consent was obtained from the patient for the publication of this case report.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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