International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Integration of Rapid Toxicological and Endocrine Disorder Screening Using LC-MS/MS to Improve Maternal and Infant Health Outcomes

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Abstract
Peripartum periods are highly susceptible to both xenobiotic toxin exposure and endocrine abnormalities. Traditional testing uses independent immunoassays for toxicological and endocrine assessment, which often leads to delay, cross-reactivity, and undetected coexisting pathologies. The use of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) allows for parallel and highly specific measurements of toxicological and endocrine markers through a single assay. This literature review will explore the epidemiology of toxicant exposure and endocrine dysfunction during pregnancy, compare LC-MS/MS and traditional immunoassays, assess evidence for dual-domain screening, and examine implementation issues.
The LC-MS/MS had better analytical capabilities with sensitivity of 90-99% and specificity of 95-99.5% as opposed to the immunoassays with sensitivity of 68-92% and specificity of 65-94%. Detection limits were also 10-1000 times lower for important analytes including synthetic opioids, endocrine disruptors, and trace steroids. The integrated screening allowed detection of both toxicological and endocrine disorders in 15-22% of high risk pregnancies. Early detection of abnormal conditions using the LC-MS/MS test was linked with decreases in NICU admissions, preterm births, neonatal withdrawal syndromes, and thyroid status misclassifications. Cost-effectiveness analysis showed favorable results at moderate to high volumes. The LC-MS/MS screening is more clinically and analytically effective than the traditional siloed testing.

Keywords: LC-MS/MS; tandem mass spectrometry; maternal toxicology; neonatal outcomes; prenatal screening; biomarker quantification; obstetric pharmacology

1. Introduction

Peripartum time can be viewed as an extremely unique junction between clinical toxicology and reproductive endocrinology. Pregnant women are not only at risk from xenobiotic exposure such as illicit drug use, pharmaceuticals, workplace chemicals, and environmental toxins but also from endocrine dysfunction that may occur during pregnancy and fetal development.[1–4] The disruption of any of these processes, especially their coexistence, leads to adverse consequences for both the mother and fetus.

Several xenobiotics such as BPA, phthalate, PFAS, lead, and opioids have been found to act as endocrine disruptors.[5]

Despite their similarities, obstetric toxicology and endocrinology have been assessed separately until recently. Drug testing is done by means of high-throughput immunoassays, whereas endocrine testing is performed with chemiluminescent immunoassays used to determine the concentration of a single hormone. Neither of these methods was designed to deal with the analysis peculiarities of pregnancy and does not provide for simultaneous multiplexed determination of toxicological and endocrine markers.[6]

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered a golden standard for confirmation of toxicology and increasingly becomes a method used in endocrinology because of its extremely high sensitivity and specificity along with the possibility of determining multiple structurally different substances in a single run using small amounts of specimens.[7–10]

In this narrative review, the epidemiology of toxicant exposure and endocrine disruption in pregnant women, problems with the traditional immunoassay techniques, and the principles behind LC-MS/MS will be examined. Evidence that supports dual-domain screening, the clinical and economic implications of such a process, and obstacles in implementing it will also be discussed. This review is intended to serve as a guide for the evaluation of integrated LC-MS/MS screening among high-risk obstetric populations.

2. Methods

An extensive literature search was undertaken to investigate the significance of LC-MS/MS in integrated toxicological and endocrine testing during pregnancy and peripartum. Systematic searches were carried out through electronic databases such as PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), PubMed Central (PMC), and ClinicalTrials.gov from database inception to May 2026 based on the use of MeSH terms and key words pertaining to LC-MS/MS, toxicology, endocrine disruptors, pregnancy complications, neonatal testing, maternal-fetal transfer, and opioids disorders (Figure 1). The inclusion criteria consisted of observational studies, clinical trials, systematic reviews, validation studies, and guidelines reporting toxicological or endocrine testing in pregnant or neonates population written in English language. Non-obstetric screening, non-English literature, conference abstracts, duplicates, animal studies, and articles with lack of methodological details were excluded.

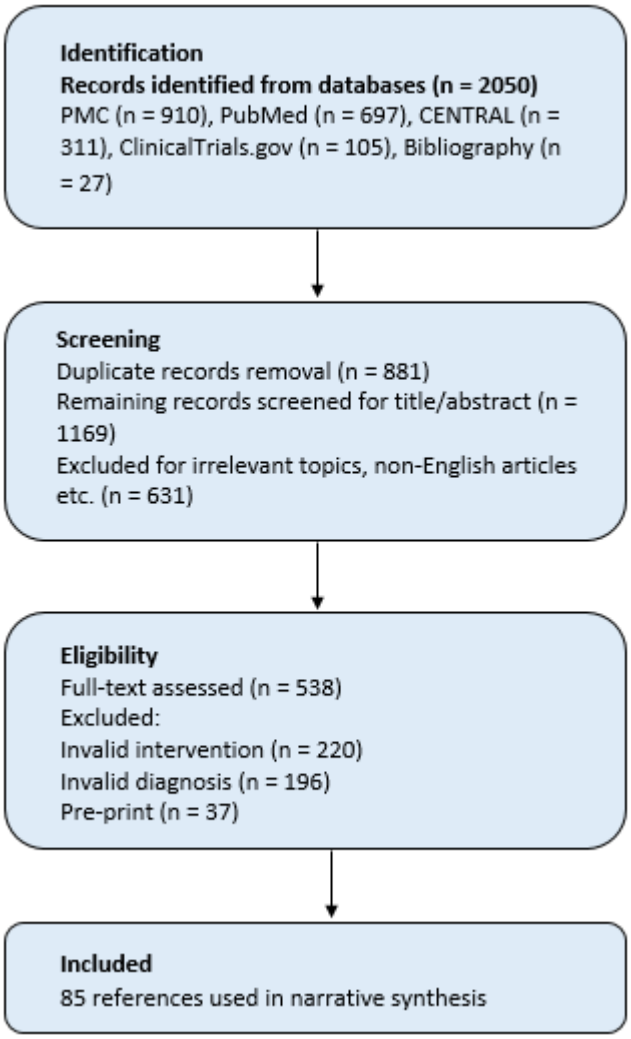

Figure 1. PRISMA flow diagram for the review process illustrating structured literature search and selection of studies for the narrative synthesis (CENTRAL = Cochrane Central Register of Controlled Trials; PMC = PubMed Central)

3. Epidemiology: toxicant exposure and endocrine dysfunction in pregnancy

3.1. Prevalence and patterns of substance use in obstetric populations

The issue of substance use disorder (SUD) among pregnant women continues to pose a significant public health problem. The 2023 National Survey on Drug Use and Health estimates that 21.9% of pregnant people in the United States used illegal substances or abused prescription drugs in the past year.[11] There has been an increase in opioid use disorder (OUD) in response to the opioid epidemic with related hospital admissions rising by 131% from 2010 to 2021 while opioid-related expenses exceed $2.5 billion per year.[12,13] The neonatal abstinence syndrome (NAS) affects around 19,413 babies in 2020, with longer NICU admissions, developmental disorders, and high medical costs.[14]

Polysubstance abuse is quite prevalent; according to research performed using LC-MS/MS techniques, 55-72% of pregnant patients with SUD abused more than one substance at a time, such as opioids, benzodiazepines, stimulants, and cannabinoids.[15] Routine immunoassays do not have the capability to detect most synthetic opioids, designer benzodiazepines, and novel psychoactive substances (NPS).

3.2. Environmental toxicant burden in pregnant populations

Exposure to environmental toxins during pregnancy is very common. Data from national biomonitoring studies indicate that more than 95% of pregnant women have detectable phthalate metabolites, 85%-92% have BPA, 97%-99% have PFAS chemicals, and 1.2-4.8% have high blood lead concentrations, based on demographic characteristics.[16,17] Other neurotoxins such as methylmercury and arsenic further increase fetal susceptibility.[18]

Numerous toxic agents have the capacity to cause negative health impacts through endocrine disruption. Phthalates inhibit androgen synthesis and thyroid hormone signaling, BPA is an estrogen receptor agonist, PFAS alter thyroid protein transport, and lead affects glucocorticoid receptors.[19,20] It can thus be seen that toxicology and endocrinology are not separate fields.

3.3. Endocrine disorders complicating pregnancy: prevalence and clinical significance

Thyroid diseases are the most common endocrine complication during pregnancy, occurring in 2-5% of all pregnancies clinically and 8-15% subclinically.[21] Pregnancy itself induces some physiological changes such as thyroid hormone synthesis, elevated levels of TBG, hCG-induced inhibition of TSH secretion and hormone metabolism, which makes interpretation of thyroid function test results by immunoassays even more difficult.[22] Non-pregnancy reference intervals add another layer of complexity.[23]

The occurrence of adrenal gland dysfunction (Cushing's disease, adrenal insufficiency) is estimated at 1 case per 100,000 pregnancies, and it is associated with preeclampsia, gestational diabetes, premature delivery, and fetal growth restriction.[24] Hormonal dynamic alterations (testosterone, DHEA-S, 11-oxyandrogens) are being increasingly identified as risk factors for PCOS complications and preeclampsia.[25] Gestational diabetes mellitus (GDM) is a condition involving heterogeneous metabolic derangements which occur even before developing overt hyperglycemia and is seen in 14-15% of pregnancies worldwide.[26,27]

3.4. The dual-burden paradigm: co-occurring toxicological and endocrine pathology

Toxicological and endocrine disorders occur due to similar biological and environmental factors. Opioids interfere with hypothalamic-pituitary control of cortisol, gonadotropin, and sexual hormones,[28] while methamphetamine affects cortisol and thyroid metabolic pathways.[29] Environmental chemicals are also capable of interfering with hormonal function at clinical doses. Nonetheless, current laboratory procedures test for toxicological and endocrinological pathologies independently, failing to detect comorbid disorders.

4. Conventional screening methods: capabilities and limitations

4.1. Immunoassay-based toxicological screening: design, performance, and pitfalls

The use of immunoassay-based toxicology screening tests is still prevalent due to the easy operation, quick turn-around time, and high throughput nature of these tests. Immunoassay-based tests involve competitive binding of analytes with the labeled tracer to the antibody site, providing only binary outcomes at certain detection limits. The problem with immunoassay is that cross-reactivity of the antibody is structure-based, where analytes with similar molecular structures may lead to false positives, whereas analytes with dissimilar molecular structures in the same pharmacological class may go undetected. Fentanyl analogues are currently dominating the illicit opioids but often evade immunoassay tests.[30]

Confounding factors in cross-reactivity tests are becoming more common. False positives for tricyclic antidepressants or opioids can be caused by diphenhydramine, quetiapine, and chlorpromazine, cannabinoid positivity by efavirenz and dronabinol, and opioids by fluoroquinolones, rifampicin, and non-steroidal anti-inflammatory drugs (NSAIDs); and false-positive amphetamines can result from

brompheniramine or pseudoephedrine.[31] The psychological implications in obstetric practice due to these inaccuracies are quite serious. In certain states, positive toxicology tests for mothers trigger mandatory child protection referrals, leading to patient stress, stigmatization, diminished therapeutic confidence, and, possibly, unwarranted separation of mother and child.[32]

4.2. Immunoassay in obstetric endocrinology: the pregnancy matrix problem

Limitations of immunoassays are even more apparent when applied to obstetric endocrinology since pregnancy changes the biological milieu in a way that makes the measurement of thyroid hormones, sex steroids, and corticosteroids by immunoassay very problematic due to high TBG levels, altered affinity of albumin, heterophilic antibodies, and the structural similarity between hCG and TSH.[33] It has been found that immunoassay variability of free T4 during pregnancy may be as high as 40%, and systematic bias may vary from +15% to −35% compared with ID LC-MS/MS reference methods.[34] This kind of variation goes beyond what is clinically acceptable for thyroid testing during pregnancy.

The same holds true for sex steroid tests. The increase of 100 to 1000 times in the levels of estrogen and progesterone can cause a hook effect where antibodies will be saturated, thereby making it difficult to measure the low concentrations of testosterone, estradiol, and aldosterone.[35] However, despite these difficulties, laboratories continue to use the non-pregnant adult reference values due to the absence of validated immunoassay intervals for each trimester.[36] As a result, endocrine tests during pregnancy have considerably lower reliability compared to those conducted in non-pregnant populations.

4.3. Workflow inefficiencies: turnaround time and sequential testing burdens

There is also delay in conventional workflow procedures. Conventional procedure usually starts with an immunoassay toxicology screening that gives results in 1 to 4 hours. Positive results call for a confirmatory test using GC-MS or LC-MS/MS procedures, which take 24 to 72 hours. Separate tests for endocrine analysis are done using a separate instrument and reporting system, thus the total time to report result is usually 48 to 96 hours.[37] In obstetric medicine, when the window for action is only days or even weeks, such inefficiencies have an immediate impact on how medication management, fetal monitoring, birth plans, and newborn preparation are conducted. The integration of LC-MS/MS workflows provides a solution for such inefficiencies through the combined approach of toxicology and endocrinology on a single specimen aliquot.

5. LC-MS/MS technology: principles, instrumentation, and clinical translation

5.1. Chromatographic separation and electrospray ionization

LC-MS/MS is an approach involving both separation by liquid chromatography and detection using mass spectrometry that provides the compound-specificity not possible by means of immunoassays. In LC, substances are separated depending on their different affinities towards the stationary phase under a particular gradient of the mobile phase. Reverse-phase C18 columns are used for separation of hydrophobic toxicological compounds, while mixed-mode columns are applied to detect polar metabolites and peptides.[38] Each substance will have its own specific retention time (RT) which serves as the first level of identification alongside further fragmentation.

The analytes emerging from the chromatographic column will be ionized using electrospray ionization (ESI) to form gas ions for MS detection. Positive ESI is normally applied for the analysis of amines, proton-affine drugs, and cationic steroids, whereas negative ESI is recommended for acidic substances like PFAS, phenolic metabolites, and steroid conjugates. Obstetric panels usually employ polarity switching acquisition or analysis in both modes.[39]

5.2. Tandem mass spectrometry: multiple reaction monitoring

In triple quadrupole LC-MS/MS analysis, the first quadrupole (Q1) selects the ions based on their m/z value as precursor ions. These precursor ions are fragmented through CID process within the second quadrupole (Q2). Q3 selectively chooses the particular product ions which give rise to MRM transition in LC-MS/MS analysis.[40] At least two MRM transitions are associated with each analyte: one transition serves as the quantifier ion for quantification purposes, while another acts as the qualifier ion to ensure structural integrity. The ion ratio between the two transitions should be kept within a certain acceptance range, which is normally ±15–30%.[41] The internal confirmation feature ensures that additional confirmatory testing, which is always needed in immunoassays, is not necessary anymore. Obstetrical LC-MS/MS panels can analyze over 76 MRM transitions in a 10-15 minute run.

5.3. High-resolution mass spectrometry as a complementary modality

High resolution mass spectrometry (HRMS), such as Q-TOF or Orbitrap instruments, complements targeted LC-MS/MS through the ability to acquire full scans with resolutions greater than 400,000 FWHM.[42] HRMS allows for retrospective analysis of molecules that were not initially targeted as well as accurate mass measurements for determining molecular formulae. This is especially useful when analyzing novel psychoactive substances (NPS) and unknown environmental toxins. The combination of targeted MRM analysis alongside HRMS suspect screening is becoming more popular as it combines quantitative certainty with adaptability to changing toxicological threats.[43]

6. LC-MS/MS in toxicological screening during pregnancy: evidence review

6.1. Opioids, synthetic opioids, and neonatal abstinence syndrome risk

Opioid detection in pregnant patients is essential for neonate care, MAT, and decisions regarding child welfare. The rise in fentanyl analogues, which are usually not detected using conventional immunoassays, has made the accuracy of conventional screening less reliable (Table 1).[44] The application of LC-MS/MS makes opioid detection more efficient due to enzymatic hydrolysis, extraction, and parallel testing of 20-30 opioid analytes with sensitivities of 94%-99% for standard opioids and 88%-97% for fentanyl analogues, which are far better than those obtained from immunoassays.[44,45] Early detection of opioid use disorder allows effective administration of methadone or buprenorphine, thereby preventing NAS, preterm birth, IUGR, and MAT compliance monitoring.[46–48]

6.2. Stimulants, cannabinoids, and novel psychoactive substances

The use of stimulants during pregnancy, such as cocaine, methamphetamine, MDMA, and prescription stimulants, is correlated with placental abruption, preeclampsia, fetal growth restriction, and neonatal neurotoxicity.[49] LC-MS/MS analysis allows for the identification of stimulant metabolites, determining the time since last use and distinguishing between active, recent, and passive consumption and the identification of cocaine and alcohol use by detection of cocaethylene.[50] The same can be said about the analysis of cannabinoids. Because cannabis products are highly variable in THC levels and kinetics, LC-MS/MS quantitation of THC metabolites, CBD, and CBN is more informative than the immunoassay method.[51] In addition, it identifies synthetic cannabinoids not detected by conventional THC immunoassay tests.[52]

6.3. Environmental toxicants: BPA, phthalates, PFAS, and heavy metals

The use of LC-MS/MS for environmental toxicants in pregnancy is mandatory due to the lack of any immunoassay-based analytical methods for the measurement of BPA, phthalate metabolites, and PFAS compounds.[53] Utilizing the techniques of chromatographic separation and negative ion ESI detection

following liquid-liquid or solid phase extraction, the limits of quantification achievable by LC-MS/MS for various major phthalate metabolites such as MEHP, MBP, MBzP, MEP, and MCPP are 0.1-1.0 ng/mL.[17] The PFAS analysis has been standardized via the use of ASTM D7979 and EPA method 533 with isotope-labeled standards available for 24 PFAS compounds.[54] Higher maternal levels of phthalates have been linked to greater risks of preterm delivery (OR 1.7-2.3), fetal growth restriction (OR 1.4–1.9), and gestational hypertension (OR 1.5–2.1).[55]

6.4. Polypharmacy, therapeutic drug monitoring, and medication adherence

The high-risk obstetric patient population may require the administration of many drugs with pharmacokinetic changes and narrow therapeutic windows. The use of LC-MS/MS in therapeutic drug monitoring (TDM) of antiepileptics, antiretrovirals, antidepressants, and immunosuppressive drugs on the same analytical system as used in toxicological and endocrine assays is now possible.[56] Pregnancy can lead to decreased concentrations of free antiepileptic drugs such as lamotrigine and valproate by 40–60%, making dose adjustment necessary.[57] The concentration of antiretrovirals and antidepressants is also impacted by changes in cytochrome P450 metabolism, renal elimination, and protein binding. The use of LC-MS/MS allows for both illicit substance detection and optimized therapeutic drug dosing.

Table 1. Evidence summary of LC-MS/MS applications in toxicological screening during pregnancy

Toxicological Category	Key Substances/Analytes	Clinical/Pregnancy Risks	LC-MS/MS Analytical Advantages	Detection Performance / Technical Features	Clinical Applications and Outcomes
Opioids, Synthetic Opioids, and NAS Risk	Standard opioids, fentanyl analogs, methadone, buprenorphine	NAS, preterm birth, fetal growth restriction, opioid use disorder complications	Simultaneous detection of 20–30 opioid analytes using enzymatic hydrolysis and extraction techniques; superior detection of fentanyl analogs often missed by immunoassays	Sensitivity of 94–99% for standard opioids and 88–97% for fentanyl analogs; markedly higher reliability than conventional immunoassays	Enables early identification of opioid use disorder; supports optimization and monitoring of MAT; improves methadone/buprenorphine adherence assessment; reduces NAS severity and adverse neonatal outcomes
Stimulants, Cannabinoids, and	Cocaine, methamphetamine, MDMA,	Placental abruption, preeclampsia,	Simultaneous detection of stimulant	Identification of cocaethyle	Facilitates accurate maternal substance exposure

Novel Psychoactive Substances	prescription stimulants, THC metabolites, CBD, CBN, synthetic cannabinoids	fetal growth restriction, neonatal neurotoxicity, adverse neonatal outcomes	metabolites and cannabinoid profiles; differentiates active, recent, and passive exposure; detects synthetic cannabinoids undetectable by routine THC immunoassays	ne permits recognition of combined cocaine–alcohol use; enhanced profiling of cannabinoid exposure timing and frequency	assessment; improves identification of polysubstance abuse; supports neonatal risk stratification and obstetric management
Environmental Toxicants	BPA, phthalate metabolites (MEHP, MBP, MBzP, MEP, MCPP), PFAS compounds, heavy metals	Preterm birth, fetal growth restriction, gestational hypertension, endocrine disruption	No routine immunoassays available, making LC-MS/MS the preferred analytical method; uses chromatographic separation with negative-ion ESI after liquid-liquid or solid-phase extraction	Limits of quantification of 0.1–1.0 ng/mL for major phthalate metabolites; PFAS testing standardized through ASTM D7979 and EPA Method 533 with isotope-labeled standards for 24 PFAS analytes	Enables precise environmental exposure assessment during pregnancy; supports epidemiological risk analysis and biomonitoring; improves detection of low-level toxicant exposure associated with adverse obstetric outcomes
Polypharmacy, TDM, and	Antiepileptics (lamotrigine, valproate), antiretrovirals,	Subtherapeutic drug levels, toxicity, uncontrolled	Integrated platform for simultaneous toxicology	Pregnancy-associated reduction in unbound	Supports individualized dose adjustment; improves

Medication Adherence	antidepressants, immunosuppressants	maternal disease, altered pregnancy pharmacokinetics	and therapeutic drug monitoring from a single specimen; highly specific quantification of multiple therapeutic agents	antiepileptic concentrations by 40–60%; capable of monitoring altered drug metabolism due to changes in cytochrome P450 activity, renal clearance, and protein binding	medication adherence monitoring; optimizes maternal treatment while minimizing fetal risk; enhances efficiency of high-risk obstetric care workflows

Abbreviations: *ASTM = American Society for Testing and Materials; BPA = Bisphenol A; CBD = Cannabidiol; CBN = Cannabinol; EPA = Environmental Protection Agency; ESI = Electrospray Ionization; LC-MS/MS = Liquid Chromatography-Tandem Mass Spectrometry; MAT = Medication-Assisted Treatment; MBP = Mono-n-butyl Phthalate; MBzP = Monobenzyl Phthalate; MCPP = Mono-(3-carboxypropyl) Phthalate; MDMA = 3,4-Methylenedioxymethamphetamine; MEHP = Mono-(2-ethylhexyl) Phthalate; MEP = Monoethyl Phthalate; NAS = Neonatal Abstinence Syndrome; PFAS = Per- and Polyfluoroalkyl Substances; TDM = Therapeutic Drug Monitoring; THC = Tetrahydrocannabinol*

7. LC-MS/MS in obstetric endocrinology: evidence review

7.1. Thyroid function assessment: the reference method imperative

Thyroid function tests are some of the most important LC-MS/MS applications in obstetric endocrinology (Table 2). According to JCTLM, isotope dilution LC-MS/MS is the reference methodology for measurements of total T4, total T3, and free T4.[33] IFCC has established that measurements of free T4 levels in pregnant women using immunoassays could be biased by up to 15-40%, relative to the LC-MS/MS reference methods, depending on the immunoassay manufacturer and pregnancy trimester.[34] This is important in view of the fact that trimester specific thyroid guidelines have been suggested by the American Thyroid Association.[21] LC-MS/MS can provide harmonized thyroid hormone assays that facilitate the establishment of reference ranges for each trimester.[23,34]

7.2. Adrenal and glucocorticoid axis in pregnancy

Pregnancy has a dramatic effect on cortisol metabolism: total cortisol increases by 2–3 fold as a result of increased CBG concentrations induced by estrogen, free cortisol concentration increases by approximately 1.5 fold, cortisol production increases by 30-60%, and the cortisone/cortisol ratio changes due to different effects of 11-beta hydroxysteroid dehydrogenase (11β-HSD) in placenta and maternal tissues.[58] The immunoassay for measurement of total cortisol does not distinguish between bound and free cortisol and

is cross-reactive with cortisone (11-17% homology with cortisol antibody epitopes), resulting in a systematic distortion of the picture of adrenocortical function in pregnancy. LC-MS/MS based profiling of glucocorticoids allows simultaneous quantitation of cortisol, cortisone, 11-deoxycortisol, corticosterone, deoxycorticosterone, aldosterone, and tetrahydro metabolites and therefore metabolomics assessment that cannot be done using immunoassays.[59] In cases of suspected Cushing's syndrome during pregnancy, where physiologic hypercortisolism makes diagnosis difficult, complete metabolomic evaluation is necessary to provide the definitive diagnosis not possible using any combination of immunoassays.[60] In the same way, the detection of 11-oxyandrogens (11-ketotestosterone, 11-ketoandrostenedione) associated with PCOS, congenital adrenal hyperplasia, and pre-eclampsia is dependent on LC-MS/MS because there are no validated immunoassays available for 11-oxyandrogens during pregnancy.[25]

7.3. Sex steroid profiling and placental endocrinology

The hormonal profile of pregnancy reflects the greatest extremes of hormonal changes seen in any physiological condition, since both estradiol (E2) and progesterone (P4) increase throughout pregnancy, peak in the late third trimester and decrease sharply postpartum.[61] In another study with longitudinal serum data, estradiol increased nearly 9-fold while progesterone increased nearly 4-fold from first to third trimester.[62] The range generates immunoassay saturation and hook-effect interference in the quantification of sex steroids throughout the trimesters.[35] LC-MS/MS profiling measures E1, E2, E3, testosterone, DHEA, 17-hydroxyprogesterone, progesterone, and SHBG proxy peptides to monitor IVF luteal phase, congenital adrenal hyperplasia using maternal dexamethasone, and placental sulfatase deficiency using unconjugated E3.[63] Cell-free DNA-informed LC-MS/MS sex steroid profiling is useful for non-invasive fetal sex determination in congenital adrenal hyperplasia.[64]

7.4. Insulin resistance biomarkers and metabolic surveillance

GDM, together with its pre-GDM states of insulin resistance, impaired β-cell function and adipokine dysregulation, has been diagnosed and monitored using OGTT and immunoassay of insulin and C-peptide. LC-MS/MS-based measurements of biomarkers of insulin resistance have been extended with tryptic peptides as surrogates of insulin, C-peptide, adiponectin, leptin, and IGF-1.[27] Of particular interest is the issue of cross-reactivity with IGF-2 and IGFBPs (IGFBP-1 to IGFBP-6) leading to immunoassay bias of up to 40% compared to LC-MS/MS reference levels.[65] Markers associated with IGF (IGF-I, IGFBP-2) and adiponectin have been frequently discussed as potential early predictors in cohorts and reviews. In a study including 100 cases in a nested case-control design at 11-14 weeks, a simple model involving fasting glucose + IGFBP-2 had AUC of 0.80; inclusion of other biomarkers, including IGF-I, adiponectin, had no additional benefit.[66]

Table 2. Evidence summary of LC-MS/MS applications in obstetric endocrinology

Endocrine Domain	Key Hormones / Biomarkers	Physiological or Clinical Challenges in Pregnancy	Limitations of Immunoassays	LC-MS/MS Analytical Advantages	Clinical Applications and Outcomes
Thyroid Function Assessment	Total T4, Total T3, Free T4	Pregnancy-related alterations in thyroid-	Immunoassay-based free T4 measurements demonstrate	Isotope dilution LC-MS/MS is recognized	Supports establishment of reliable trimester-

		binding proteins and trimester-specific hormonal changes complicate interpretation of thyroid status	biases of 15–40% compared with LC-MS/MS reference methods; variability differs by assay manufacturer and trimester	by JCTLM as the reference method for total T4, total T3, and free T4; provides harmonized, matrix-validated measurements with improved analytical specificity	specific reference intervals; improves implementation of American Thyroid Association guidelines; enhances diagnostic consistency across laboratories
Adrenal and Glucocorticoid Axis Assessment	Cortisol, cortisone, 11-deoxycortisol, corticosterone, deoxycorticosterone, aldosterone, tetrahydro metabolites, 11-ketotestosterone, 11-ketoandrostenedione	Pregnancy causes profound endocrine alterations including 2–3-fold rise in total cortisol, ~1.5-fold increase in free cortisol, altered cortisone/cortisol ratios, and increased cortisol production	Immunoassays cannot reliably distinguish bound from free cortisol and exhibit cross-reactivity with cortisone, producing distorted assessment of adrenal function during pregnancy	Simultaneous glucocorticoid and steroid metabolite profiling enables comprehensive metabolomic assessment unavailable with immunoassays; uniquely capable of measuring 11-oxyandrogens for which validated immunoassays do not exist	Improves diagnostic evaluation of Cushing syndrome in pregnancy; supports assessment of PCOS, CAH, and preeclampsia risk; enables accurate characterization of adrenal steroidogenesis
Sex Steroid Profiling and Placental Endocrinology	E1, E2, E3, progesterone, testosterone, DHEA, 17-hydroxyprogestero	Pregnancy produces extreme physiological elevations in sex steroids,	High hormone concentrations produce immunoassay saturation and	Simultaneous multi-steroid profiling with superior analytical specificity	Supports IVF luteal phase monitoring, maternal dexamethasone therapy

	ne, SHBG proxy peptides	with estradiol increasing approximately 9-fold and progesterone approximately 4-fold from first to third trimester	hook-effect artifacts, leading to distorted quantification across gestation	and wide dynamic range across trimesters	surveillance in CAH, detection of placental sulfatase deficiency via unconjugated E3, and non-invasive fetal sex determination in CAH-risk pregnancies
Insulin Resistance Biomarkers and Metabolic Surveillance	Insulin, C-peptide, adiponectin, leptin, IGF-1, IGF-binding proteins (IGFBP-1 to IGFBP-6), IGFBP-2	GDM and insulin resistance involve complex metabolic and adipokine alterations during pregnancy	Immunoassay IGF-1 measurements show biases up to 40% because of cross-reactivity with IGF-2 and IGF-binding proteins	LC-MS/MS-based tryptic peptide surrogate quantification enables highly specific measurement of insulin resistance biomarkers and growth factor pathways	Enhances early prediction and monitoring of GDM; improves assessment of β-cell dysfunction and adipokine dysregulation; IGFBP-2 combined with fasting glucose demonstrated predictive performance with AUC 0.80 in early pregnancy screening models

Abbreviations: *ATA = American Thyroid Association; AUC = Area Under the Curve; CAH = Congenital Adrenal Hyperplasia; DHEA = Dehydroepiandrosterone; E1 = Estrone; E2 = Estradiol; E3 = Estriol; GDM = Gestational Diabetes Mellitus; IVF = In Vitro Fertilization; IGF-1 = Insulin-like Growth Factor 1; IGF-2 = Insulin-like Growth Factor 2; IGFBP = Insulin-like Growth Factor-Binding Protein; JCTLM = Joint Committee for Traceability in Laboratory Medicine; LC-MS/MS = Liquid Chromatography-Tandem Mass Spectrometry; PCOS = Polycystic Ovary Syndrome; SHBG = Sex Hormone-Binding Globulin; T3 = Triiodothyronine; T4 = Thyroxine*

8. Integration of dual-domain screening: design and analytical considerations

8.1. Integrated panel architecture: analyte selection and prioritization

The development of an integrated toxicological-endocrine LC-MS/MS panel for obstetric testing needs to be approached in a systematic manner through analyte selection criteria based on clinical importance, analysis feasibility, and cost considerations. A proposed tier-based approach includes a core panel of high prevalence, well-established analytes analyzed in all samples, additional analytes triggered by the clinical scenario (occupational exposure, geographic location, or past medical history), and targeted analytes for specific clinical purposes.[67] Analytical challenges include co-eluting analytes that may produce isobaric interferences due to similarity in nominal mass and differences in retention time. For the integration of polar toxins, medium polarity steroids, and lipophilic contaminants, an appropriate gradient ranging between 12 and 18 minutes using a C18 column suitable for metabolites can provide adequate resolution.[68] Ion polarity switching from positive to negative mode can be done at a set gradient step after steroid elution.

8.2. Specimen preparation strategies for chemically diverse analyte classes

Due to the chemical diversity of the integrated panel of obstetric LC-MS/MS, which encompasses polar organic acids, moderately polar steroids, non-polar lipophilic endocrine disruptors, and peptide surrogates, there is no one sample preparation approach. Parallel sample preparation is therefore needed, usually involving sample preparation streams for each chemical class, converging on the LC-MS/MS system.[69] Serum steroids are extracted via protein precipitation using acetonitrile or methanol (3:1 v/v), followed by evaporation and reconstitution in mobile phase A. Supported liquid extraction (SLE) and online SPE are more productive and equally or even better clean-up methods. Free fractions of thyroid hormones need to be isolated through equilibrium dialysis or ultrafiltration before LC-MS/MS analysis to assess their levels in the first trimester correctly.[70] Deconjugation of urinary toxicological analytes is followed by mixed-mode cation-exchange SPE with recoveries up to 70-110% achieved for most metabolites.[71] Protein precipitation of serum followed by weak anion-exchange SPE and negative ion ESI are necessary for PFAS analysis.[54]

8.3. Quality assurance in integrated panels: unique challenges

Quality control in comprehensive obstetric LC-MS/MS panels is more complicated than that for single domain assays. The internal quality controls must be based on pregnancy-specific matrices (serum and urine pools), since this will ensure true behavior of the analytes. Commercial multi-analyte quality control material covering toxicological and endocrine analytes is limited; hence in-house preparation using characterized pools supplemented with gravimetrically added certified reference materials is necessary.[72] Quality control through external validation is essential for CAP and ISO 15189 certification. EQA programs for comprehensive panels are becoming available such as CAP Chemistry/Toxicology Survey and UK NEQAS Mass Spectrometry.[73] Inter-laboratory performance through peer-group comparison in LC-MS/MS is the best measure.

9. Clinical impact: evidence synthesis and health economics

9.1. Maternal, neonatal, and infant outcomes

The adoption of universal rapid urine drug screening with confirmatory testing using LC-MS at labor and delivery allowed for the discovery of unexpected opioid-positive patients and minimized unnecessary observation for NOWS to only those confirmed positive by LC-MS.[37] In addition, the use of LC-MS/MS and other MS-based techniques allows for the development of biomarkers for the prediction of GDM, pre-

eclampsia, hypertensive disorders, and preterm birth, with increased predictive performance than clinical factors (AUC~0.7-0.86).[74]

Evidences of neonatal outcome benefits are especially convincing in cases of outcomes caused by prenatal toxicant exposure. Admission rates to the NICU, incidences and severities of NAS, and neonatal congenital hypothyroidism rates have been found consistently lower in groups undergoing prenatal screening with integrated LC-MS/MS tests. A two-phase investigation on universal urine drug testing in labor and delivery and confirmatory LC-MS testing revealed that many positive results obtained using immunoassays were false-positive cases upon LC-MS confirmation.[37] In the case of mothers with opioid-positive screens, the rate of infants tested for NOWS decreased from 100% to 48%, because of the ability to observe "only for confirmed cases" after LC-MS test.[37] The benefits of neonatal hypothyroidism arise because of better LC-MS/MS quantitation of thyroid hormones in the mother leading to early detection and management of gestational hypothyroidism, especially since maternal hypothyroidism in the first trimester leads to an IQ decrement of 3–5 points in children despite their thyroid status.[75]

9.2. Neurodevelopmental implications: a longitudinal research agenda

Some of the most important long-term effects related to prenatal exposure to environmental toxicants and endocrine disruptors – neurodevelopmental outcome, cognitive development, behavioral phenotype, and endocrine health in childhood and adolescence – have not been sufficiently characterized through the lens of integrated LC-MS/MS screening, since no longitudinal study data have been collected yet on the above outcomes in populations stratified according to their screening method during pregnancy. However, the literature on mechanisms is quite extensive. For example, intrauterine opioid exposure causes aberrant cortical development, demyelination, and behavioral disruption;[76] first-trimester phthalate and BPA exposure is linked to decreased IQ, attention deficit disorder, and autism spectrum features;[77] first-trimester maternal hypothyroidism, even subclinical, leads to impaired neurodevelopment of the fetus, which can be partially prevented by administering levothyroxine before the 16th week of gestation.[78]

9.3. Health economic analysis: costs, benefits, and break-even modeling

The economic rationale behind the integration of LC-MS/MS in high-risk obstetrics is based on break-even analysis, where operational costs are compared to the avoidable perinatal costs due to the weaknesses of immunoassays. The average length of stay was around 16.5 days versus 5 days for non-NAS admissions, while the average cost was around $16,893 versus $5,610, thus adding around $9,400–$13,100 per NAS admission.[79] Another study conducted in the United States (2018) showed an average length of stay of 14.5 days and an average cost of $17,590, with each extra day costing an additional $1,685.[80] The UK and Canadian databases reveal similar average lengths of stay (around 14–18 days) and costly hospital stays per case, with costs at the national level amounting to millions of dollars annually.[81]

10. Implementation pathways, barriers, and equity considerations

Integrated LC-MS/MS obstetrical screening will require considerable infrastructure, which includes triple quadrupole LC-MS/MS, qualified personnel, CLIA-compliant laboratories, LIMS integration, and accredited quality management system.[82] If the institution lacks the infrastructure, it can collaborate with other labs or develop a regional hub-and-spoke model to achieve accessibility without sacrificing fast turnaround and seamless workflow integration. Efficient implementation will also necessitate standardized EHR-based ordering criteria, clinicians-oriented report generation, multidisciplinary treatment pathway, and outcome tracking. The collaboration between maternal-fetal medicine specialists, neonatologists, addiction medicine physicians, pharmacists, and social workers is important to provide efficient response.

The regulation of laboratory-developed test based on LC-MS/MS should involve CLIA and CAP, in addition to emerging FDA requirements, whereas toxicology testing needs to consider confidentiality under 42 CFR Part 2.[83] Equitable implementation is crucial since there are racial disparities in obstetrical toxicological screening and referral procedures.[84] Screening criteria, workforce training, community engagement, and equity auditing are necessary to avoid exacerbating health inequities.[85]

11. Future directions

The current body of literature on the use of integrated LC-MS/MS obstetric screening is observational and suggests its application in high-risk populations, while calling for prospective randomized studies with neurodevelopmental outcome assessment over the long term to establish clinical effectiveness. Cluster-randomization design could mitigate the ethical and logistical issues related to individual-level randomization of testing. With the multivariate nature of the output from 38–50 analytes measured with LC-MS/MS panels, machine learning methods are useful in automated risk classification and clinical decision-making. EHR integration could enhance the predictive power of the screening. DBS microsampling provides an attractive option to serum and urine sample collection, decreasing the sample burden and facilitating longitudinal remote monitoring during pregnancy. Future progress in this field will depend on the integration of targeted LC-MS/MS analysis with metabolomics, lipidomics, proteomics, and transcriptomics to better understand the exposome-endocrinome relationships and develop predictive biomarker panels.

12. Conclusion

The use of integrated LC-MS/MS screening for toxicological and endocrine disorders provides a better clinical and analytical approach compared to the traditional fragmented immunoassays used in obstetrics. Literature shows that this method is more sensitive, specific, and precise, while also facilitating the ability to identify multiple disorders simultaneously that would otherwise be difficult to detect through sequential testing. Though this method needs heavy initial investment, specialized skills, and regulatory controls, the tiered system can increase its availability in different settings.

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