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A systematic review

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Electrochemical biosensors for monitoring of selected pregnancy hormones during the first trimester: A systematic review

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ABSTRACT

The hormones human chorionic gonadotropin, progesterone, estrogen and four of its metabolites (estradiol, estrone, estriol, estetrol), as well as relaxin play an essential role in the development of the fetus during the first trimester. Imbalances in these hormones during the first trimester have been directly linked to miscarriages. However, frequent monitoring of the hormones is limited by the current conventional centralized analytical tools that do not allow a rapid response time. Electrochemical sensing is considered an ideal tool to detect hormones owing to its advantages such as quick response, user-friendliness, low economic costs, and possibility of use in point-of-care settings. Electrochemical detection of pregnancy hormones is an emerging field that has been demonstrated primarily at research level. Thus, it is timely with a comprehensive overview of the characteristics of the reported detection techniques. This is the first extensive review focusing on the advances related to electrochemical detection of hormones linked to the first trimester of pregnancy. Additionally, this review offers insights into the main challenges that must be addressed imminently to ensure progress from research to clinical applications.

1. Introduction

Approximately 15–20% of clinically recognized pregnancies end in miscarriage, defined as a loss of pregnancy before the twentieth week of gestation [1]. In almost half of the cases, the cause for the miscarriage is unknown [2]. Furthermore, 1–2% of women suffer from recurrent miscarriage defined as three or more consecutive miscarriages [2,3]. Some of the known factors associated with recurrent miscarriage include earlier miscarriages, increased age, and genetic factors such as chromosomal abnormalities in the fetus. Furthermore, anatomical causes like uterine anomalies, environmental exposures, and endocrine problems namely endocrine diseases and hormonal imbalance are also known to cause recurrent miscarriage [2–7]. Of all miscarriages of clinically recognized pregnancies, 80% occur during the first trimester [2].

Pregnancy hormones play an essential role in ovulation, early implantation and attachment of the embryo [8]. In addition, normal hormonal balance is also important in maintaining pregnancies during the first trimester [9]. The hormones, human chorionic gonadotropin (hCG), progesterone, estrogen and four of its metabolites (estradiol, estrone, estriol, estetrol), as well as relaxin play an essential role in the development of the fetus during the first trimester. Imbalances in these hormones during the first trimester have been directly linked to miscarriages. This includes extrauterine pregnancy, impending miscarriage, placental insufficiency, and fetal death. For this reason, these four hormones are the point of focus in this review [10–14].

Human chorionic gonadotropin is important in driving the hemochorial placentation during pregnancy as well as stimulating the corpus luteum in the ovaries [15,16]. Progesterone is crucial for preparation of the endometrium for implementation and maintaining pregnancy through several endocrinological functions [9,17]. Estrogens ensure the growth, lining and differentiation of uterine glands, stimulation of growth and functionality of the placenta along with regulation of gonadotropin secretion; thus, estrogens are necessary for both female fertility and pregnancy [18,19]. During the first trimester, relaxin helps implantation of fetus to the uterine wall and placental growth [20]. The relative trajectory of the four hormones during the first trimester is displayed in Fig. 1.

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Despite the awareness of the critical roles of these hormones during the first term of pregnancy, the literature on the specific etiology behind their behavior and the related miscarriages are not yet fully comprehended. There is a lack in knowledge about fluctuations of hormone levels during the course of pregnancies. Pregnant women can have hormonal fluctuations that are out of balance and still lay within the normal reference ranges that are relatively large [24]. To account for the differences from one pregnancy to another, doubling time for hCG has been used as a better reference when tracking hormone fluctuations during pregnancy progression [1]. To make individualized treatment options, it is important to understand the fluctuations of the hormones on an individual basis [25]. Frequent monitoring of hormone levels can contribute to our basic knowledge about fluctuations during pregnancy.

Currently, most data on hormonal concentrations are obtained via immunochemical methods. These methods are well developed and precise in measuring hormonal concentrations. However, they depend on sampling at specific sites and analysis of samples in centralized laboratories. The time from sampling to result is consequently long, the process is inconvenient for parents, and delays decision-making regarding possible medical intervention [18,25]. As the sampling is troublesome and personnel-heavy, the frequency of sampling is low and thus data about fluctuations of hormones during the course of pregnancy is limited.

Electrochemical biosensors are emerging within the field of fast, high-throughput detection of specific health markers and are ideal to monitor hormonal levels, due to their high sensitivity, selectivity, portable options, rapid response time and low-cost [26–28]. Thus, electrochemical biosensors have the potential to become powerful tools for frequent and fast monitoring of hormones for the benefit of a more comprehensive understanding of hormonal fluctuations during the course of pregnancy. On-the-spot electrochemical monitoring of pregnancy hormones would especially be convenient for people experiencing recurrent miscarriages. However, the realization of electrochemical monitoring of pregnancy hormones for medical use is yet to be accomplished [29,30].

To date, there is only one extensive review by Bahadir and Sezgintürk from 2014 on electrochemical biosensors for detection of pregnancy related hormones [31]. This is the first review in the field. Additionally, a review conducted by Khanwalker et al. from 2019 covers several hormones associated with fertility including two relevant hormones for pregnancy development (progesterone and estradiol) [18]. Furthermore, electrochemical biosensing for hormone detection is a field that has developed extensively since the publication of these two general reviews. Here, we provide the first focused overview of electrochemical detection of four selected hormones that are essential during the first trimester of pregnancy. The review provides a comprehensive description of the different electrochemical approaches utilized to detect the hormones and gives a status on the development in the field. Finally, this review points to crucial directions for future research to realize hormonal monitoring with electrochemical sensing in clinical applications to ensure healthy progression of pregnancies.

2. Methodology

2.1. Screening for articles

Two separate screenings were carried out. First, a screening for existing reviews on electrochemical detection of pregnancy related hormones was performed to investigate the amount of research done in this field. The screening included the following search terms: hCG, progesterone, estrogen, and relaxin cross combined with electrochemical detection, electrochemical biosensors, voltammetry, amperometry, electrochemical impedance spectroscopy and EIS. The date of publication was not considered, but only human related studies which used a relevant electrochemical method were included. Two reviews were found [18,31], see Supplementary Fig. S1.

The second screening was performed to find other relevant articles on electrochemical detection of pregnancy hormones not included in the two abovementioned reviews (Fig. 2). The search for articles was performed via PubMed, including Medline, and PubMed Central. Furthermore, research was done in Embase, Cochrane and The Royal Danish Library in order to attain a broad screening The screening was carried out in a way that articles would always have key terms present in either the abstract, title or methods; "hormone" [Abstract] OR "hormone" [Title] OR "hormone" [Methods - Key terms] AND "electrochemical method keyword" [Abstract/Title/Methods - Key terms] on PubMed Central and "hormone" [Title/Abstract] AND "electrochemical method keyword" [Title/Abstract] on PubMed. Inclusion criteria for this second screening were that the studies had to be human related, used a relevant electrochemical method and hormone and that all necessary information were accessible including hormone, electrochemical method, linear range, and LOD. Only English language articles were included. In addition to the necessary information, the material of the working electrode, as well as functionalization and the type of substrate used for
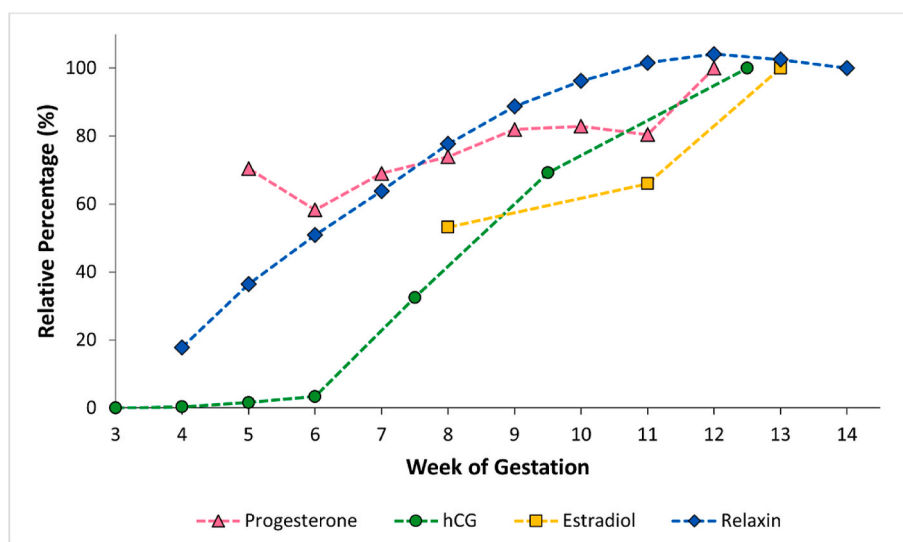

Fig. 1. Relative percentage of progesterone, hCG, estradiol and relaxin levels for every week in the first trimester beginning with week 3. Constructed by data derived from Ku et al., 2018, Lyngbye, 2010, Tulchinsky et al., 1972 and Conrad, 2011, respectively [20–23].
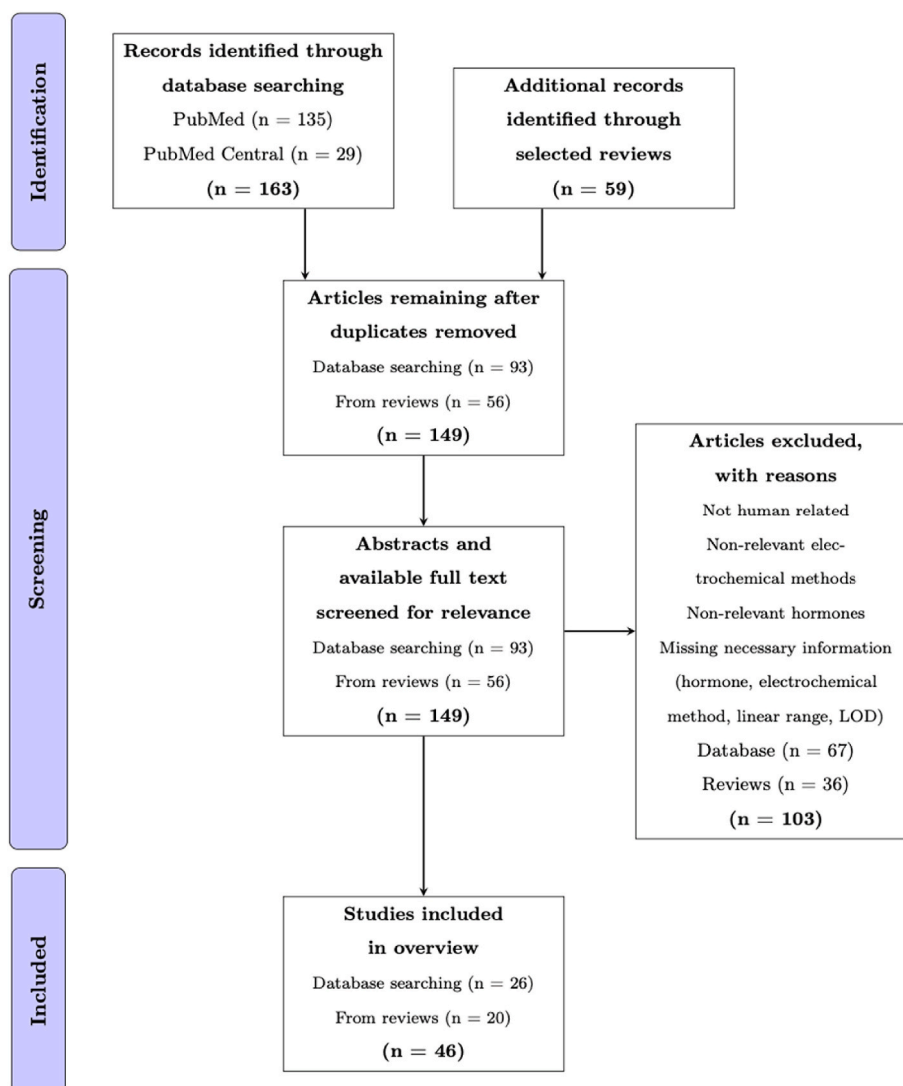
Fig. 2. Flowchart of the screening process for studies on electrochemical detection of selected hormones.

the hormone analysis were extracted from the articles and compiled into tables.

2.2. Screening output

From the two existing reviews 20 articles were derived and from our systematic screening 26 articles were acquired, resulting in a total of 46 articles. Of these 46 articles, 7 included studies on more than one relevant hormone. Thus, a total of 52 hormonal measurements divided into 11 studies for hCG, 10 studies for progesterone and 31 studies on estrogens were included in this review. These have been compiled into Tables 2–4, giving an overview of articles published on PubMed and PubMed Central on electrochemical detection of hCG, progesterone, estrogen, estradiol, estrone, estriol, estetrol and relaxin using amperometry, voltammetry or electrochemical impedance spectroscopy (EIS). This review seeks to increase a focus on the importance of hormone monitoring during pregnancy and further provides an extensive review on the development of electrochemical biosensors with specificity to pregnancy hormones. Distinct key terms for the screening were carefully selected to secure a thorough inclusion of reports on electrochemical sensing of pregnancy hormones. However, we acknowledge that even though great effort has been made to avoid shortcomings when making this review, some reports have potentially been excluded during the screening process due to lack of key terms present in the title, abstract or methodology.

Although there are few reviews based on the electrochemical detection of pregnancy related hormones, many reviews such as [32–34] compile articles for electrochemical sensing of progesterone and estradiol. Several reviews present articles on such issues as steroid hormone concentrations in wastewater and work to progress detection for these problems. However, overviews of the applicability of these detection methods for monitoring and the improvement of pregnancy success rates are lacking. This review includes hormones relevant for the first trimester of pregnancy and provides a comparison of the linear ranges found within the studies to observed physiological concentration ranges of hormones. This review has great relevancy as it provides a close focus and insight for future research in this direction.

3. Detection strategies of pregnancy hormones using electrochemical biosensors

3.1. Basic principles for electrochemical detection

Electrochemical sensors are commonly carried out in a three-electrode configuration: a working electrode where an electrochemical reaction occurs, a reference electrode with a known, defined potential and a counter electrode that closes the circuit and acts as a source/sink of electrons. The output reading is typically current, potential or

impedance recordings from the sample. The setup enables detection of redox activity of molecules, where peak potentials often is utilized as an identification source, while the intensity of the peak corresponds to the quantity of the molecule. The ability to measure redox activity in samples has been utilized to develop several strategies for selective and quantitative detection of analytes like hormones [35–37].

The direct oxidation of hormones is a common strategy employed for electrochemical-based monitoring of hormones. In this approach, the hormone of interest is directly oxidized at the electrode surface to produce an electroactive product, which can be measured electrochemically. However, direct oxidation may be challenging because of the complex nature of the biological matrix, which can lead to non-specific binding and electroactive interference from other species in the sample.

To address these issues, indirect detection methods have been developed that use specific recognition elements such as Molecular Imprinted Polymers (MIPs), antibodies, or aptamers to selectively bind the hormone of interest. The recognition element is then coupled to the electrode surface, allowing for sensitive and selective detection of the target hormone. Indirect detection methods can offer higher specificity compared to direct detection methods. However, the development of recognition elements can be time-consuming and expensive, and their performance can be impacted by the presence of interfering substances.

In the following, the main electrochemical detection strategies used for measuring hormones are described and schematically summarized in Fig. 3.

3.2. Direct redox detection of hormones

The most direct way of detecting hormones using electrochemical sensors is by measuring the reduction and/or oxidation activity of the hormone. This would however require that the hormone of interest is redox-active and thus capable of exchanging electrons with the electrodes without mediation of e.g., enzymes. This method is simple, however; it requires thorough investigation of other interfering redox compounds in the sample matrix. Depending on the complexity of the matrix, it may be required to pretreat the samples before detection [18, 38].

3.3. Immuno-biosensors for hormone detection

Immuno-biosensors are based on the specific recognition of antigens by antibodies immobilized on the working electrode. The read-out is typically depending on suppressing of signal when applying a known redox-compound on the sensor after incubation with the sample or by tagging the antigen with a redox-tag that can be identified electrochemically. As this approach usually consists of up to several washing steps, immune-biosensors may be powerful in selective detection of the hormone of interest [39].

3.4. Biosensors based on Molecular Imprinted Polymers for hormone detection

The process of molecular imprinting is to produce polymers with specific cavities within the polymer matrix that corresponds to the target molecules. In this way the negative imprints of the molecules will function as selective recognition sites for the molecule. Polymerization is often conducted on the working electrode, allowing specific detection of the molecule even in the presence of similar molecule structures [40].

3.5. Functionalization of biosensors with aptamers for hormone detection

Aptamers are short, single-stranded DNA or RNA molecules that can selectively bind to a specific like hormones. Functionalization of electrodes using aptamers implies immobilizing aptamers on working electrodes [41]. The aptamer sequences enable high specific molecular recognition and affinity towards the analyte by creating a specific three-dimensional conformation that folds around the target of interest [42,43]. Aptamers usually consist of sizes between 35 and 100 nucleotides which ensure a high surface density of receptors and thereby enabling high sensitivity [41].

3.6. Magnetic nanoparticles for increased efficacy

Magnetic nanoparticles in electrochemical detection serve to increase the efficacy of the sensor. Magnetic nanoparticles are usually functionalized by coating with a conductive material that allows for surface modifications such as antibodies or other analyte recognition techniques as listed above [44]. Functionalized magnetic nanoparticles
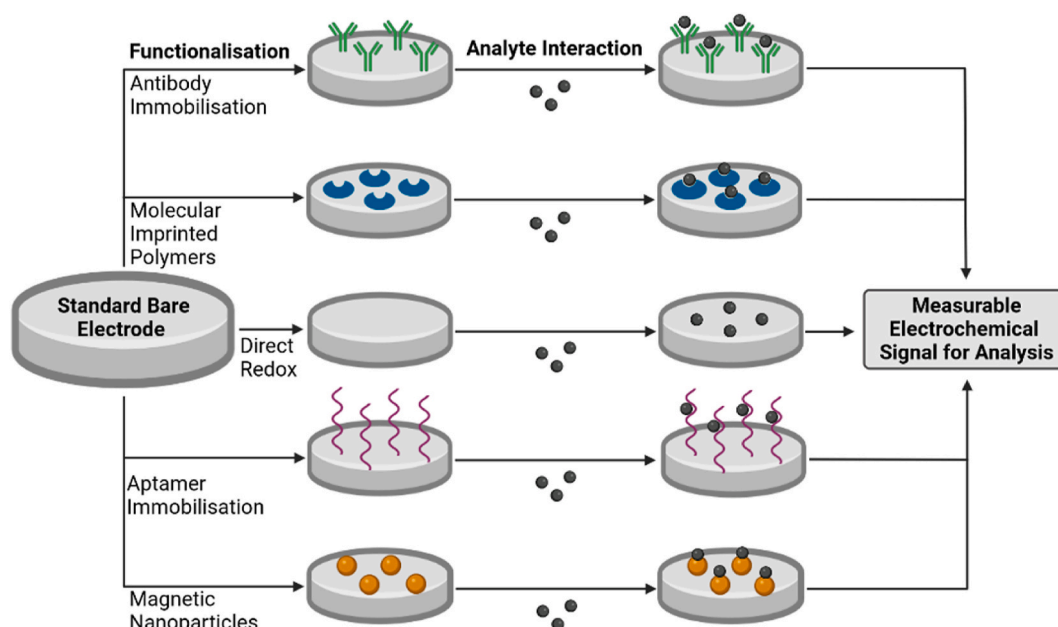

Fig. 3. Schematics illustrating the main sensor functionalization techniques. Created with BioRender.com.

contribute with increased surface area of the electrode, faster electron transfer, reduced noise and better sensitivity [45]. Magnetic nanoparticles have also been utilized on platforms that were entirely non-conductive, thus serving as the electrode themselves [46].

4. Detection of selected pregnancy hormones

If electrochemical biosensors are to be applicable in clinical settings, it is of great importance to be able to measure the entire physiological range of the individual hormones, during the first trimester. Table 1 summarizes the functions, relevant physiological concentrations and common symptoms related to malfunctions of the selected hormones. These parameters are crucial for the assessment of the performance of the electrochemical biosensors for hormones developed to date. In the following, we provide an overview of the limit of detection (LOD), linear range, detection strategy and the type of matrix that have been utilized in the respective studies.

3.7. Human chorionic gonadotropin

The glycoprotein hormone, human chorionic gonadotropin, partially consists of the non-covalently bound α- and β-subunits as seen in Fig. 4. It is primarily produced by and secreted from the placental syncytiotrophoblast cells [49].

Human chorionic gonadotropin plays a crucial role in driving the hemochorial placentation during pregnancy. Throughout the initial weeks of pregnancy, hCG stimulates the corpus luteum, maintaining the production of progesterone [15,16]. The physiological hCG levels increase drastically from week four to five, reaching their peak at week 11–14 and decreases through the rest of the pregnancy as seen in Fig. 5. In the first trimester, hCG levels range between 11 pM and 660 nM and have a doubling time of approximately two days [22]. Low levels of hCG can be attributed to a blighted ovum eventually leading to miscarriage [50]. Furthermore, reduced rate of increased hCG levels is also seen in extrauterine pregnancy, impending miscarriage and placental insufficiency [22]. Abnormally high hCG level during pregnancy are related to having a higher risk of complications, including gestational

Table 1
Overview of concentrations, functions and symptoms due to malfunctions of selected hormones.

Concentration for non-pregnant	Concentration for pregnant	Method used for Detection	Function During Normal Pregnancy	Symptoms of Dysfunction due to High Concentration	Symptoms of Dysfunction due to Low Concentration	Reference
Human Chorionic Gonadotropin						
Fertile: <11 pM Post-menopausal: <22 pM	1. Trimester: 11–660,000 pM 2. Trimester: 52,800–121,000 pM 3. Trimester: 13,200–105,600 pM	Immunochemical Mass spectrophotometry	Drives the hemochorial placentation Stimulates the corpus luteum in the ovaries Maintains the production of progesterone	Gestational hypertension Miscarriage Premature birth, small for date babies Risk of fetal malformations Premature placental abruption Neonatal respiratory distress syndrome	Extrauterine pregnancy Impending miscarriage Placental insufficiency	[15,16,22]
Progesterone						
Follicular phase: <5000 pM Luteal phase: 20,000–100,000 pM Just before menstruation: <10,000 pM	1. Trimester: 30,000–140,000 pM 2. Trimester: 60,000–300,000 pM 3. Trimester: 200,000–500,000 pM	Immunochemical	Sustainability of early gestation Control of uterine contractility Decrease of the mother's immunological response preventing the excretion of the embryo Stimulation of morphological changes of the cervix to increase cervical competence		Miscarriage Preterm labor	[9,11,17, 21,22]
Estrogen						
Estradiol: Early follicular phase: 90–180 pM Pre-ovulatory phase: 700–1500 pM Luteal phase: 280–1000 pM Post-menopausal: <80 pM	*Estradiol:* 1. Trimester: 140–11,630 pM 2. Trimester: 2484–60,925 pM 3. Trimester: 157–123,741 pM *Estriol:* 1.+2. Trimester: Increases 20–25% per. Week 3. Trimester: Increases tenfold	Immunochemical	Modulation of the uterus - stimulates growth Maintains functionality of the placenta Helps regulate gonadotropin secretion Supports fetal development *Estradiol:* Acts as a growth hormone for reproductive organs Ensures proper environment for oocytes in the ovaries Prepares the uterine- and mammary tissues to respond to progesterone	Miscarriage	Miscarriage – Spontaneous or habitual abortion. *Estriol:* In fetus: Neural tube defects, chromosomal abnormalities, fetal death, placental sulfatase deficiency	[18,19,22, 47,48]
Relaxin						
Luteal phase: Averagely 13.33 pM Otherwise: Extremely low to non-existent	1. Trimester 93.33–172.67 pM 2. Trimester 66.67–116.67 pM 3. Trimester 66.67–116.67 pM	Immunochemical	Vasodilatory, counteracting cardiovascular complications Increases insulin reception, preventing gestational diabetes Relaxes the cervix and ruptures the placental membrane upon labor Assists implantation and placental growth	Preterm labor Miscarriage Hypoglycemia	Fibrosis Gestational diabetes Cardiovascular complications	[12,20]

Table 2

Overview of reports on electrochemical detection of Human Chorionic Gonadotropin (hCG). Abbreviations: LOD—limit of detection, DPV—differential pulse voltammetry, EIS—electrochemical impedance spectroscopy, PBS - phosphate-buffered saline.

Technique	Sample Matrix	Working Electrode Material	Functionalization of Electrode	Linear Range Converted Value (Linear Range Original Value & Unit)	LOD Converted Value (LOD Original Value & Unit)	Reference
Amperometry	PBS (pH 6.9)	Gold nanotubes array	Antibody	0.22–220 pM ($0.1 - 100$ mIU/mL^{-1})	0.17 pM (0.08 mIU/mL^{-1})	[52]
Amperometry	PBS	Glassy carbon	Antibody	$13.62 - 1089.92$ pM ($0.5 - 40.00$ ng/mL^{-1})	0.92 pM (0.034 ng/mL^{-1})	[53]
Amperometry	PBS (pH 7)	Glassy carbon	Antibody	$5.5 - 27.5$ pM ($2.5 - 12.5$ mIU/mL)	3.08 pM (1.4 mIU/mL)	[54]
Amperometry	Synthetic Urine (pH 6.8)	Graphene	Antibody	$16.89 - 153.13$ pM ($0.62 - 5.62$ ng/mL)	16.89 pM (0.62 ng/mL)	[38]
Amperometry	PBS (pH 7.5)	Glassy carbon	Antibody	$55 - 880$ pM ($25 - 400$ mIU/mL)	26.4 pM (12 mIU/mL)	[55]
DPV	Blank Human Serum Sample	Carbon	Antibody	$6.6 - 206725.2$ pM (3.0 mIU/mL $-$ 93.966 IU mL^{-1})	0.792 pM (0.36 mIU/mL)	[27]
DPV	HCl solution	Carbon (Single-walled carbon nanotube)	Antibody	NA	0.052 pM (2.4 pg/mL)	[56]
DPV	PBS	Gold	Antibody	$0.027 - 5.44$ pM & $5.44 - 1653.95$ pM^{aa} ($0.001 - 0.2$ & $0.2 - 60.7$ ng/mL)	0.008 pM (0.3 pg/mL^{-1})	[28]
DPV	PBS (pH 7)	Glassy carbon	Antibody	$0.48 - 660$ pM ($0.2 - 300$ mIU/mL)	0.17 pM (0.08 mIU/mL)	[57]
DPV	PBS (pH 7.0)	Graphite	Antibody	$0.22 - 22$ pM ($0.1 - 10$ mIU/mL)	0.132 pM (0.06 mIU/mL)	[58]
EIS	PBS Human urine Synthetic urine	Carbon nanotube	Antibody	$0.27 - 272.48$ pM^{bb} ($0.01 \cdot 10^{-9}$ g/cm$^{-3} - 100 \cdot 10^{-9}$ g/cm^{-3})	0.27 pM ($0.01 \cdot 10^{-9}$ g/cm^{-3})	[51]

[a] As for the two linear ranges, the smaller range is before binding of antibody and antigen, and the larger is after a change in pH and the antibodies and antigens having bound to each other.

[b] Measurements were performed in both PBS, synthetic urine and real urine, and this range covers the linear range for all 3 measurements.

Table 3

Overview of reports on electrochemical detection of progesterone. Abbreviations: LOD—limit of detection, CV – Cyclic Voltammetry, DPV—differential pulse voltammetry, EIS—electrochemical impedance spectroscopy, SWV – Square wave voltammetry, PBS - phosphate-buffered saline.

Technique	Sample Matrix	Working Electrode Material	Functionalization of Electrode	Linear Range Converted Value (Linear Range Original Value & Unit)	LOD Converted Value (LOD Original Value & Unit)	Reference
Amperometry	PBS	Carbon	Antibody	500–50,000 pM ($5 \cdot 10^{-10} - 5 \cdot 10^{-8}$ mol/dm^3)	1000 pM ($1 \cdot 10^{-9}$ mol/dm^3)	[69]
Amperometry	PBS (pH 7.0)	Graphene	NA	$25,000 - 1.79 \cdot 10^9$ pM ($0.025 - 1792.5$ µM)	4280 pM (4.28 nM)	[64]
CV	KCl buffer (pH 6)	Gold	Imprinted polymers	$3 \cdot 10^{-6} - 0.031$ pM (0.001–10 fg/mL)	$8 \cdot 10^{-6}$ pM (2.5 ag/mL)	[70]
CV	PBS	Indium-tin-oxide	Imprinted polymers	NA	3.18 pM (1.0 pg/mL^{-1})	[71]
DPV	PBS (pH 7.5)	Carbon	Aptamer	$10 - 10^6$ pM ($0.01 - 1000$ nM)	1.86 pM	[63]
EIS	NA	Gold	Aptamer	$31,800 - 190,803$ pM ($10 - 60$ ng/mL)	2862.05 pM (0.9 ng/mL)	[72]
EIS	PBS	Gold disk	Antibody	Physiological range: $3180.05 - 95401.6$ pM^{aa} ($1 - 30$ ng/mL)	26,140 pM (8.22 ng/mL)	[18]
SWV	Bovine Serum	Gold	Antibody	$254.4 - 22260.4$ pM ($8 \cdot 10^{-2}$ ng/mL$^{-1} - 7$ ng/mL^{-1})	254.4 pM (0.08 ng/mL^{-1})	[73]
SWV	KCl buffer	Glassy carbon	Imidazole functionalized graphene oxide	$220,000 - 1.4 \cdot 10^7$ pM ($0.22 - 14.0$ µmol/L^{-1})	216.24 pM (68 nmol/L^{-1})	[74]
Voltammetry	PBS	Carbon	Nanostructured bismuth film electrode	$100,000 - 700,000$ pM ($0.1 - 0.7$ µmol/L^{-1})	NA	[75]

[a] The 'linear range' is denoted as physiological range, since the article notes their method to be linear across the physiological range.

hypertension, miscarriage, premature birth, and babies that are small for gestational age. Additionally, at extremely elevated values the risk of fetal malformations, premature placental abruption and neonatal respiratory distress syndrome is high [22]. If the hCG levels decrease with a half-life of one day, it often is an indication of fetal death [22]. Thus, hCG is a key diagnostic marker and indicator for pregnancy progression in early pregnancy [15,51].

Eleven studies performing electrochemical detection of hCG have been found, including the ones derived from the reviews (Table 2). None of the studies cover the entire physiological range of hCG. All of the studies used antibodies as a functionalization strategy for selective detection. The majority of the studies tested the sensors in spiked phosphate-buffered saline (PBS) matrix. However, one study by Teixeira et al. tested matrices consisting of PBS, synthetic and human urine [51]. Teixeira et al. describes the analytical performance of the immunosensor to be fairly similar when applied to synthetic urine and PBS. Measurements in urine from a pregnant volunteer showed promise as the sensor was able to determine the hCG concentrations in a range corresponding to the pregnancy status of the volunteer [51]. Another study by Cao et al.

performed testing on blank human serum samples, showing promise in having a LOD of 0.79 *pM* and a linear range of 6.6 pM–206.7 nM (converted from 1.0 mIU/mL to 100.0 IU mL^{-1}) [27]. This is the broadest range for detection of hCG in this review. Due to hCG having by far the broadest physiological range of the selected hormones, it seems feasible that there would be specifically technical problems in the development of sensors capable of detecting the full relevant range of hCG.

3.8. ProgesteroneProgesterone is a C21-steroid hormone that primarily consists of a pregnane skeleton where two oxo-substituents, two methyl groups and one carbonyl group are attached as seen in Fig. 6 [59]

Progesterone has regulatory functions in the uterus, ovaries, mammary glands and furthermore partakes in regulation of the central nervous- and cardiovascular system [60]. Progesterone stimulates morphological changes of the cervix, contributes to sustain gestation by controlling relaxation of the myometrial smooth muscle cells, and decreases the mother's immunological response which prevents excretion

Table 4

Overview of reports on electrochemical detection of estrogens. Abbreviations: LOD—limit of detection, CV – Cyclic Voltammetry, DPV—differential pulse voltammetry, EIS—electrochemical impedance spectroscopy, LSV – Linear sweep voltammetry, SWV – Square wave voltammetry, PBS - phosphate-buffered saline. *** The 'linear range' is denoted as physiological range, sincethe article notes their method to be linear across the physiological range.

Technique	Sample Matrix	Working Electrode Material	Functionalization of Electrode	Linear Range Converted Value (Linear Range Original Value & Unit)	LOD Converted Value (LOD Original Value & Unit)	Reference
Amperometry	PBS	Gold	Antibody	Linear response up to ~ 440,529 pM (1200 pg/mL^{-1})	22.02 pM (6 pg/mL)	[81]
Amperometry	Human urine Human serum	Carbon	Antibody	$3.67 - 917.78$ pM ($1 - 250$ pg/mL^{-1})	2.82 pM (0.77 pg/mL^{-1})	[82]
Amperometry	PBS	Gold	Antibody	NA	36987.7 pM (10 ng/mL)	[83]
Amperometry	PBS (pH 7.4)	Glassy carbon	Antibody	$36.71 - 66079.3$ pM ($0.01 - 18$ ng/mL)	12.11 pM (3.3 pg/mL)	[84]
CV	KCl buffer	Carbon paste	Molecularly imprinted polymers	$4 - 6000$ pM ($4.0 \cdot 10^{-12} - 6.0 \cdot 10^{-9}$ M)	1.18 pM ($1.18 \cdot 10^{-12}$ M)	[45]
CV	KCl buffer (pH 6)	Gold	Molecularly imprinted polymers	$4 \cdot 10-6 - 3.67$ pM ($0.001 - 1000$ fg/mL)	0.00003 pM (9 ag/mL)	[70]
CV	PBS (pH 6.0)	Graphite paste	Octoxynol-9	$4 \cdot 10^7 - 1.2 \cdot 10^8$ pM ($4 \cdot 10^{-5} - 1.2 \cdot 10^{-4}$ M)	$1.4 \cdot 10^6$ pM ($1.4 \cdot 10^{-6}$M)	[85]
CV	PBS (pH 7.0)	Carbon	Pyrrole monomers	$183.55 - 2.569.75$ pM ($50 - 700$ ng/mL)	36,710 pM (10 ng/mL)	[86]
CV	PBS (pH 7.4)	Gold	CYP3A4 enzyme	Linear response up to ~ 150,000 pM (0.15 μM)	103 pM ($1.03 \cdot 10^{-10}$ mol/L)	[87]
DPV	BRB (pH 7.0)	Glassy carbon	Cobalt-poly (methionine)	$596,000 - 9.9 \cdot 10^6$ pM ($0.596 - 9.90$ μmol/L)	34,000 pM ($3.4 \cdot 10-8$ mol/L)	[88]
DPV	BBS (pH 10.5)	Silver/silver chloride	NA	$5000 - 2 \cdot 10^6$ pM ($5 \cdot 10^{-9} - 2 \cdot 10^{-6}$ M)	NA	[89]
DPV	BBS (pH 10.5)	Silver/silver chloride	NA	$1000 - 1.5 \cdot 10^6$ pM ($10^{-9} - 1.5 \cdot 10^6$ M)	NA	[89]
DPV	PBS (pH 7.0)	Glassy carbon	Gold nanoparticles	$10,000 - 2.2 \cdot 10^8$ pM ($0.01 - 220$ μM)	1000 pM (0.5 nM)	[90]
DPV	PBS (pH 7.2)	Graphite	Antibody	NA	55.06 pM (15 pg/mL)	[91]
DPV	PBS (pH 7.2)	Platinum	Antibody	$91.76 - 1835.33$ pM ($25 - 500$ pg/mL)	183.55 pM (50 pg/mL)	[92]
DPV	PBS (pH 6.2)	Glassy carbon	Carbon black	$150,000 - 3.5 \cdot 10^6$ pM ($0.15 \cdot 10^{-6} - 3.5 \cdot 10^{-6}$ mol/L)	92,000 pM ($9.2 \cdot 10-8$ mol/L)	[93]
DPV	PBS (pH 6.5)	Glassy carbon	Aptamer	$12 - 60,000$ pM (12 pM – 60 nM)	1.5 pM	[94]
DPV	PBS (pH 7.0)	Glassy carbon	Magnetic molecularly imprinted polymers	$25,000 - 10^7$ pM ($0.025 - 10.0$ μmol/L)	2760 pM (2.76 nmol/L)	[95]
DPV	PBS (pH 7.4)	Carbon & AgCl ink on paper wax	Antibody	$3671.07 - 367,107$ pM ($0.01 - 100$ ng/mL)	36.71 pM (10 pg/mL)	[26]
DPV	PBS (pH 7.4)	Gold	Antibody	$8.26 - 8259.91$ pM ($2.25 - 2250$ pg/mL)	NA	[96]
EIS	PBS (pH 7.4)	Gold	Receptor	NA	10^6 pM (10^{-6} M)	[97]
EIS	PBS	Gold disk	Antibody	Physiological range: $3.67 - 1835.54$ pM *** ($1 - 500$ pg/mL)	8.62 pM (2.35 pg/mL)	[18]
EIS	PBS (pH 7.0)	Gold	Receptor	$0.1 - 1000$ pM ($1.0 \cdot 10^{-13} - 1.0 \cdot 10^{-9}$ M)	0.1 pM ($1.0 \cdot 10^{-13}$ M)	[98]
EIS	PBS (pH 7.4)	Glassy carbon	Aptamer	$0.001 – 1.0 \cdot 10^6$ pM ($1.0 \cdot 10^{-15} - 1.0 \cdot 10^{-6}$ M)	0.001 pM (10−15 M)	[99]
EIS	PBS (pH 7.4)	Gold	Receptor	$0.0013 - 13.58$ pM ($3.7 \cdot 10^{-4} - 3.7$ ng/L)	1.35 pM ($3.7 \cdot 10^{-4}$ ng/L)	[100]
SWV	BRB (pH 11)	Carbon paste	Magnetic nanoparticles	$1.0 \cdot 10^6 – 1.0 \cdot 10^7$ pM ($1.0 - 10$ μM^{-1})	300,000 pM (300 nM^{-1})	[101]
SWV	BRB (pH 12)	Carbon paste	Magnetic nanoparticles	$100,000 - 10^6$ pM ($0.1 - 1.0$ μM^{-1})	50,000 pM (50.0 nM^{-1})	[101]
SWV	NaOH (pH 12.0)	Boron-doped diamond	NA	$200,000 - 2 \cdot 107$ pM ($2.0 \cdot 10^{-7} - 2.0 \cdot 10^{-5}$ mol/L−1)	170,000 pM ($1.7 \cdot 10−7$ mol/L−1)	[102]
SWV	PBS (pH 7.0)	Glassy carbon	NA	$500,000 - 1.5 \cdot 107$ pM ($5.0 \cdot 10^{-7} - 1.5 \cdot 10^{-5}$ M)	180,000 pM (180 nM)	[103]
SWV	PBS (pH 7.0)	Glassy carbon	NA	$10^6 - 7.5 \cdot 10^7$ pM ($10^{-6} - 7.5 \cdot 10^{-5}$ M)	620,000 pM (620 nM)	[103]
SWV	PBS (pH 7.0)	Glassy carbon	NA	$2 \cdot 10^6 - 5.0 \cdot 10^7$ pM ($2 \cdot 10^{-6} - 5.0 \cdot 10^{-5}$ M)	840,000 pM (840 nM)	[103]

of the embryo [9,11,17,21]. In the first trimester, the physiological progesterone levels range between 30 and 140 nM as seen in Fig. 7 [22]. Several studies suggest that inadequate levels of progesterone are associated with higher risk of miscarriage during early pregnancy [11, 21,61]. A common etiological association to low progesterone levels is a compromised function of the corpus luteum due to irregularities in the

development of the dominant follicle [21]. Low levels of progesterone can cause anovulatory cycle, luteal and placental insufficiency as well as threatened and recurrent miscarriage [62]. According to Ku et al. a lower mean level of serum progesterone ranging between 21.6 and 38.2 nM is observed in nonviable pregnancies [21]..

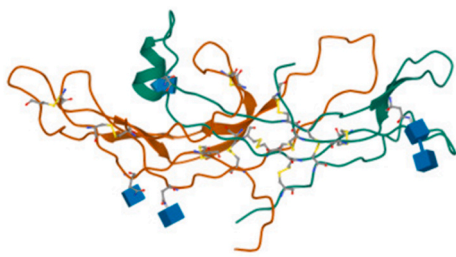Ten studies, performing electrochemical detection of progesterone,

Fig. 4. Protein structure of Human Chorionic Gonadotropin obtained via the Protein Data Bank [49].

have been included in this review including the ones from the review by Bahadir and Sezgintürk, 2014, as seen in Table 3. Two of the studies report detection that covers the entire physiologically relevant range of progesterone. Most of the studies primarily used antibodies or aptamers to functionalize the working electrodes. All studies tested the sensors in spiked PBS or blood samples diluted in PBS. The studies by Samie et al. [63] and Govindasamy et al. [64] particularly show promise as they cover the entire physiologically relevant levels with linear ranges of 10 pM – 1.0 μM and 25 nM–1.790 mM, respectively. Both studies performed electrochemical detection of progesterone in human blood samples diluted in PBS. Samie et al. created a very effective aptasensor (GQDs-NiO-AuNFs/f-MWCNTs nanocomposite), using NiO–Au hybrid nanofibers with graphene quantum dots to create nano-architecture which is then combined with multiwalled carbon nanotubes. This structure allows for many carboxylic functional groups, allowing for a high upper limit of aptamer loading. Govindasamy et al. shows a promising linear range which they attribute to the nanocomposite modified electrode, that had a superior electrocatalytic ability comparative to bare and control electrodes. Acknowledgement should also go to the studies on electrochemical detection of progesterone [65–68] that due to different exclusion criteria were not included in this review. These are still relevant in the overall status on development of electrochemical sensing of progesterone.

4.3. Estrogens Estrogen is a C18-steroid hormone that primarily consist of a hydroxyl and/or ketone substituted pregnane skeleton. Within estrogens, the three major endogenous estrogens are estrone, estradiol and estriol. During pregnancy a fourth estrogen, estetrol, is produced [62,76]. The chemical structures are seen in Fig. 8.

The functions of estrogens include the modulation of uterine growth and lining, along with differentiation of uterine glands. Estrogens also stimulate growth and maintain functionality of the placenta, support fetal growth and help regulate gonadotropin secretion [18,19]. Estrogens play an important role in maintaining pregnancy, but the exact nature of their role is still unclear. The four metabolites, estrone, estradiol, estriol and estetrol, have been demonstrated to have various effects: stimulation of growth in females' reproductive organs, progesterone receptor expression, promotion of uterine blood flow and cervical softening [22,47,77,78]. The production of estrogens during pregnancy increases a thousandfold compared to non-pregnant women and peaks around week 35 of gestation [22]. Studies on the role of estrogens in pregnancy have shown that both abnormally low and high estrogen levels cause miscarriage [19,22,79,80]. In the fetus, low estriol levels are related to neural tube defects, chromosomal abnormalities, or fetal death and placental sulfatase deficiency [22].

Table 4 summarizes 31 published studies investigating electrochemical detection of different estrogen metabolites. Some of the studies have reported the development of sensors proven to detect the full physiologically relevant range of the selected metabolites. For estradiol, 3 studies cover the physiological range during the first trimester [84,94, 99]. For estriol, one study covers the physiological range and lastly [89], no studies cover the physiological range of estrone. The strategies of
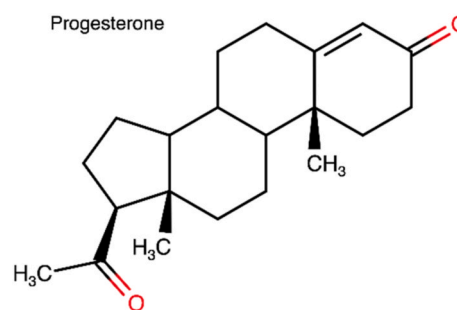

Fig. 6. Molecular structure of progesterone. Created in ACD/ChemSketch, version 2021.1.2, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada, www.acdlabs.com.
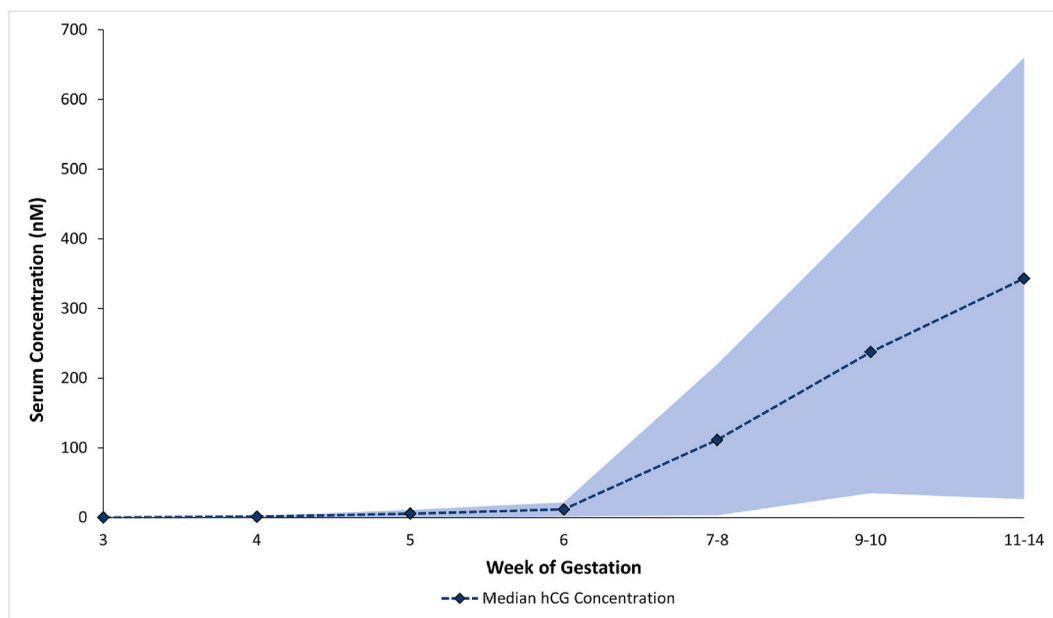

Fig. 5. Serum concentration of hCG every week in the first trimester beginning with week 3. Constructed by data from Lyngbye, 2010 [22].
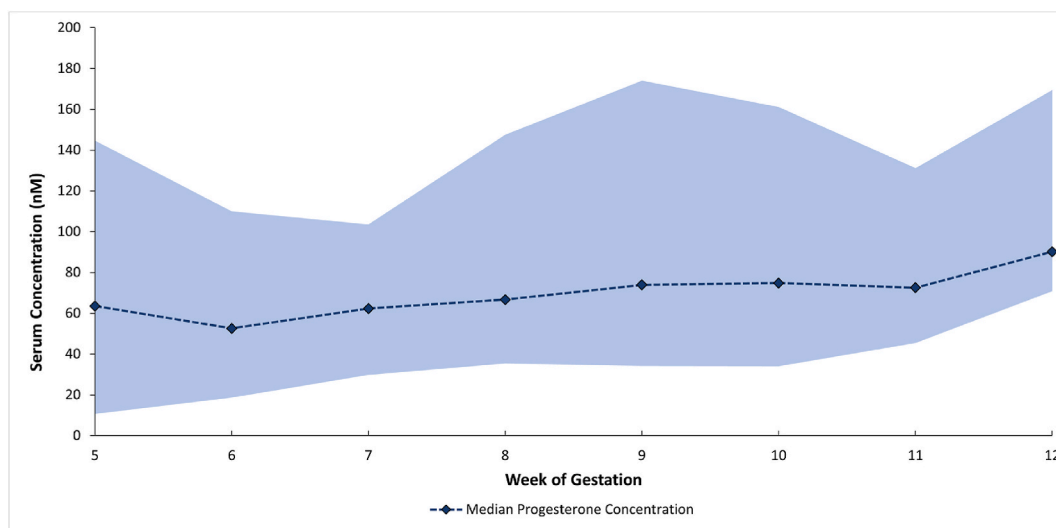
Fig. 7. Serum concentration of progesterone in every week of the first trimester beginning with week 5 generated by data from Ku et al., 2018 [21].
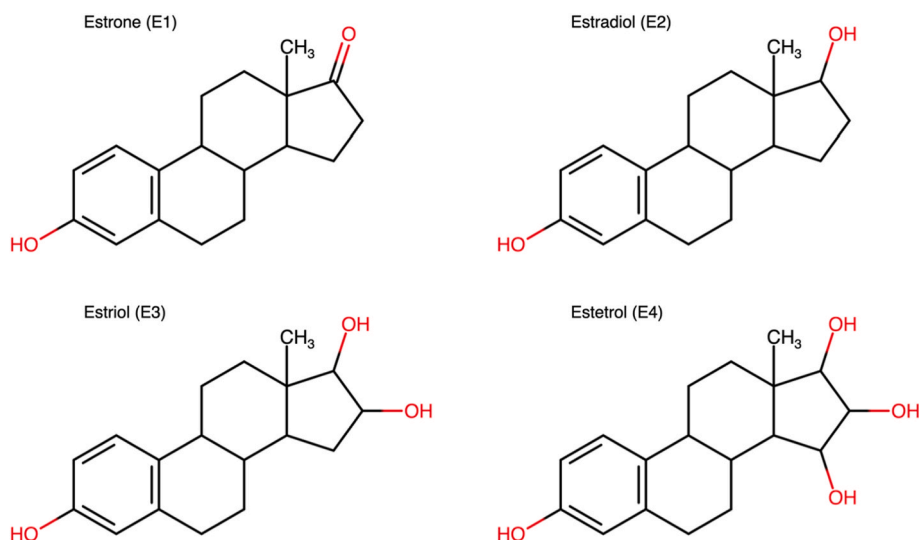

Fig. 8. Chemical structure of endogenic estrogens. The chemical structures of the estrogens are very similar, however the number of hydroxyl groups differs [62]. *Created in ACD/ChemSketch, version 2021.1.2, Advanced Chemistry Development, Inc. (ACD/Labs), Toronto, ON, Canada,* https://www.acdlabs.com

functionalization utilized are diverse, varying between antibodies, aptamers, molecularly imprinted polymers, receptor proteins and magnetic nanoparticles. Although almost no studies cover the physiological range of estrogens, there is still much potential in these electrochemical methods. When considering that the four metabolites are almost identical in their molecular structure, it could be speculated that several of the electrochemical detection methods are applicable to more than one type of sub-metabolite.

3.9. Relaxin

Relaxin is a peptide hormone consisting of two peptide chains, A and B, that are connected via three disulfide bonds [104], Fig. 9. Relaxin is secreted from the corpus luteum in the ovaries and placenta and is mostly known for its role in incitement of parturition by rupturing the placental membrane and relaxing the cervix [12].

Relaxin stimulates implantation of the fetus to the uterine wall and placental growth [20]. Furthermore, it also has vasodilatory effects, as it impacts the smooth muscle cells of many

Veins, counteracting the cardiovascular imbalance and risks of

Fig. 9. Structure of human relaxin obtained via the Protein Data Bank [105].

myocardial infarct that a pregnancy presents [106]. Circulating relaxin also has an impact on insulin reception, and speculation goes that it assists in the blood glucose homeostasis [107]. Due to this impact of relaxin on insulin receptors, it has a relation to progesterone which promotes insulin resistance [108][.During the first trimester, relaxin is at its highest at a mean of 197.88 *pM* (1.18 ng/mL) as seen in Fig. 10. Levels of relaxin, measured at about 300.18 *pM* (1.79 ng/mL) or higher during the first trimester [12] has been speculated to be an indicator of prematurity risk, resulting in the cervix relaxing too soon, causing premature childbirth (labour between week 20 and 37), along with prematurely bursting the placental membrane potentially resulting in a miscarriage [14].

To the best of our knowledge, no studies have been reported on electrochemical detection of relaxin. It could be speculated that an electrochemical biosensor with specific functionalization to relaxin is achievable when considering that this has been possible for other peptide hormones such as hCG and progesterone. Electrochemical detection of relaxin is not only useful during pregnancy but also as a potentially new and better indicator during parturition. We strongly encourage further research into the etiology and monitoring of relaxin as well as to the development of fast methods that allow frequent relaxin monitoring.

It is important to keep in mind that hormone levels can vary based on various factors such as the individual's health and the pregnancy itself. These ranges should therefore be viewed as general guidelines. The wide range of hormone levels makes it difficult to use them for diagnostic purposes. Thus, it is essential to understand that the acceptable hormone concentrations are individual and should be determined on a case-by-case basis, since factors such as binding affinity and receptor count can vary greatly from person to person [110]. Although an average range can be established, the most reliable indicator of a stable pregnancy is the percentage increase or decrease or doubling time in hormones as this metric tends to be more consistent across a population.

4. Discussion and future perspectives

The significance of electrochemical sensing for hormone detection lies in its ability to provide rapid, sensitive, and cost-effective analysis of hormones in various matrices. Electrochemical sensors can directly measure the concentration of hormones in a sample without the need for complex sample preparation or labeling steps, providing results in real-time for high-throughput applications. They have high sensitivity compared to immunochemical methods, enabling the detection of

hormones at low concentrations and making them useful for sensitive and quantitative analysis.

However, there are several challenges associated with the analysis of hormones using electrochemical detection. Hormones are often present in complex matrices, such as blood, urine, or saliva, which can contain other substances that can interfere with the electrochemical detection of hormones. Hormones are present at low concentrations in biological samples, making it challenging to achieve the high sensitivity required for accurate detection. Hormones are also often sensitive to temperature, pH, and other environmental conditions which makes it difficult to maintain their stability during the analysis process. Additionally, hormones can be structurally similar and thereby complicate high selectivity in the detection process. Electrochemical sensors must be specifically designed to target the hormone of interest to avoid cross-reactivity with other similar compounds.

4.1. The effect of lipophilicity and hydrophilicity on hormone detection

The lipophilicity and hydrophilicity of biomarkers can significantly affect their detection using electrochemical sensors [111]. Proper adsorption of the biomarker on the electrode surface affects the biomarker binding and the final blocking of the electron transfer [112]. It is important to tailor the functionalization strategy of electrochemical sensors to the specific properties of the biomarker being detected, including its lipophilicity or hydrophilicity. The lipophilic nature of biomarkers may potentially cause interference during their electrochemical detection in real patient samples. For instance, a lipophilic hormone could bind to lipids in a sample, leading to an excessive signal and misleading results. Undesired interference due to specific affinities to surfaces or to other molecules in real samples could be a possible explanation for the limited success in electrochemical detection of pregnancy hormones in non-buffer solutions. The use of appropriate functionalization layers can significantly improve the detection performance and sensitivity of electrochemical sensors for biomarker detection.

4.2. The effect of phenolic passivation on signal output

Some hormones, such as estrogen and progesterone, have phenolic groups in their structure, making them possible targets for electrochemical sensing. However, the presence of phenols can also lead to the fouling of electrode surfaces and decrease the sensitivity of
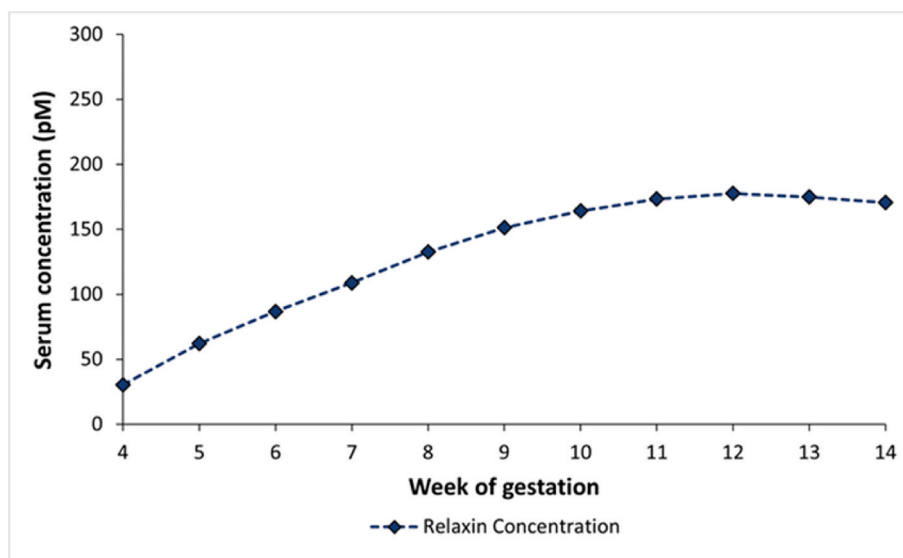

Fig. 10. Serum concentration of circulating relaxin in every week of the first trimester beginning with week 4. Data originates from a graph presented in Conrad, 2011 [20]. The data have subsequently been extrapolated by the use of WebPlotDigitizer [109].

electrochemical sensors. This phenomenon is attributed to the adsorption of phenolic compounds onto the electrode surface, which creates a passivation layer that hinders electron transfer and leads to electrode fouling [113]. Several strategies have been developed to mitigate the fouling of phenolic compounds in electrochemical sensing. One such approach involves the modification of the electrode surface with specific functional groups that can interact with phenolic compounds and minimize their adsorption [114]. Another strategy is the use of membrane-based separation techniques to separate the analyte from interfering compounds before detection [115].

Despite the challenges faced, electrochemical biosensors hold promise as attractive alternatives to conventional immunochemical methods due to their several advantages in hormonal analysis, including direct measurement, high sensitivity, and on-the-spot testing. These features offer significant potential for use in point-of-care diagnostics in the future.

4.3. The importance of mapping individual normal hormone levels

The normal hormone levels in pregnant women span broadly during the first trimester. The normal range is established based on measurements conducted decades ago and recent advances are scarce. We have yet to gather data on hormone levels linked to the specific backgrounds of the women, covering age, ethnicity, environment etc. Absolute numbers cannot be used for diagnostic or prognostic purposes based on the data available today. Thus, single hormone measurements on samples from pregnant women are not sufficient to deter potential miscarriages. The main learning from the broad normal span of the hormone levels is that each hormone needs to be normalized to the baseline and trajectory of development for the individual pregnancy.

To account for individualized hormone levels, electrochemical sensors may help achieve dense readings during each pregnancy. The vision of implementing electrochemical biosensors into healthcare systems holds promise due to the prospect of frequent point-of-care measurements. Together with better understanding of the progression of hormone levels during first trimester, the point-of-care electrochemical monitoring of pregnancy hormones may provide early warning signs if intervention is needed or provide assurance to women with recurrent miscarriages.

5. Conclusions

Approximately 80% of all miscarriages occur during the first trimester. Although it is established that abnormal fluctuations in hormone concentrations are either a consequence or a cause of adverse pregnancy outcomes, this field could benefit from frequent longitudinal measurements on individual pregnancies. This review summarizes types of electrochemical biosensors used for hormone analysis and provides an overview of their characteristics for medical applications. Comparison between different electrochemical monitoring of pregnancy hormones shows promise for future utilization due to a more user-friendly, fast and on-the-spot testing compared to today's use of immunochemical methods. Implementation of electrochemical monitoring of pregnancy hormones during the first trimester will be of special value for women who are at risk due to predisposing factors. Finally, we strongly encourage further research into exploring electrochemical detection of relaxin.

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.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2023.124396.

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