

Next-Gen Sensors for Hormonal Precision – Measurement of Progesterone and Oestradiol Concentration in Blood Concentration in Blood

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Detecting hormones in females is critical for optimizing reproductive health, accurately diagnosing hormonal disorders, and ensuring comprehensive mental and physical well-being. Current methods for hormone detection often face limitations, as they tend to be invasive, time-consuming, and costly, highlighting the need for novel techniques to address these challenges. Aptamers are short, single-stranded DNA or RNA molecules that bind specifically to target molecules, such as proteins or small compounds, with high affinity and specificity. In this study, we hypothesize that hormones can dock into the major groove of aptamers, enabling their detection using aptamers. My research focuses on two specific hormones, oestradiol and progesterone. First, we employed a systematic approach to model aptamer sequence in primary, secondary, and tertiary forms for aptamer 3D structure modeling. we used Vfold2D to transfer aptamer structures from primary to secondary by uploading the aptamer sequence and saving the dot-bracket notations. To transition from secondary to tertiary structures, we utilized the vfold3D platform, importing the sequences along with their dot-bracket notations and configuring the number of clusters to one. The final step involved molecular docking simulations of the hormones using the HDock software to study the interactions at the tertiary structural level. Based on the docking analysis, aptamer SH-ssDNA and B10-P were selected as the most appropriate candidates based on the molecular docking binding energy. The application of this research lies in developing precise biosensors for hormonal disorders. By studying aptamer-hormone interactions, this approach could be extended to create hormone diagnostic tools for reproductive health disorders and hormone-responsive cancers.

1. INTRODUCTION

Detecting estradiol, a significant estrogen produced in the ovaries, is essential across various medical fields and research, including reproductive health, endocrinology, and oncology. Several methods exist to measure estradiol levels, each offering distinct advantages and limitations. The Enzyme-Linked Immunosorbent Assay (ELISA) is usually utilized because of its high sensitivity, affordability, and ease of use, though it may suffer from cross-reactivity with other hormones.(1) Often considered the gold standard, radioimmunoassay (RIA), another greatly sensitive technique that can also be used, requires specialized equipment and the handling of radioactive substances. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) stands out as the most sensitive and specific technique, capable of precisely quantifying deficient concentrations of estradiol; however, it is costly and demands advanced instrumentation.(2)

Accurately measuring estradiol levels is crucial to diagnosing and treating conditions such as hormonal imbalances, infertility, and certain cancers, making these techniques necessary in clinical practice.

While precise detection of estradiol is vital for diagnosing, managing, and treating various medical conditions, several challenges can make the process difficult. A significant concern is cross-reactivity with other steroid hormones, such as estrone and estriol, which can lead to inaccurate and misleading results. Some other methods have also been known to lack the necessary sensitivity to detect low estradiol levels, particularly in specific patient groups, and issues like non-specific binding can compromise accuracy. Proper sample handling and storage of estradiol is critical, as innocent storage can lead to degradation and false outcomes. Cost and accessibility also create challenges, particularly for advanced methods with accuracy

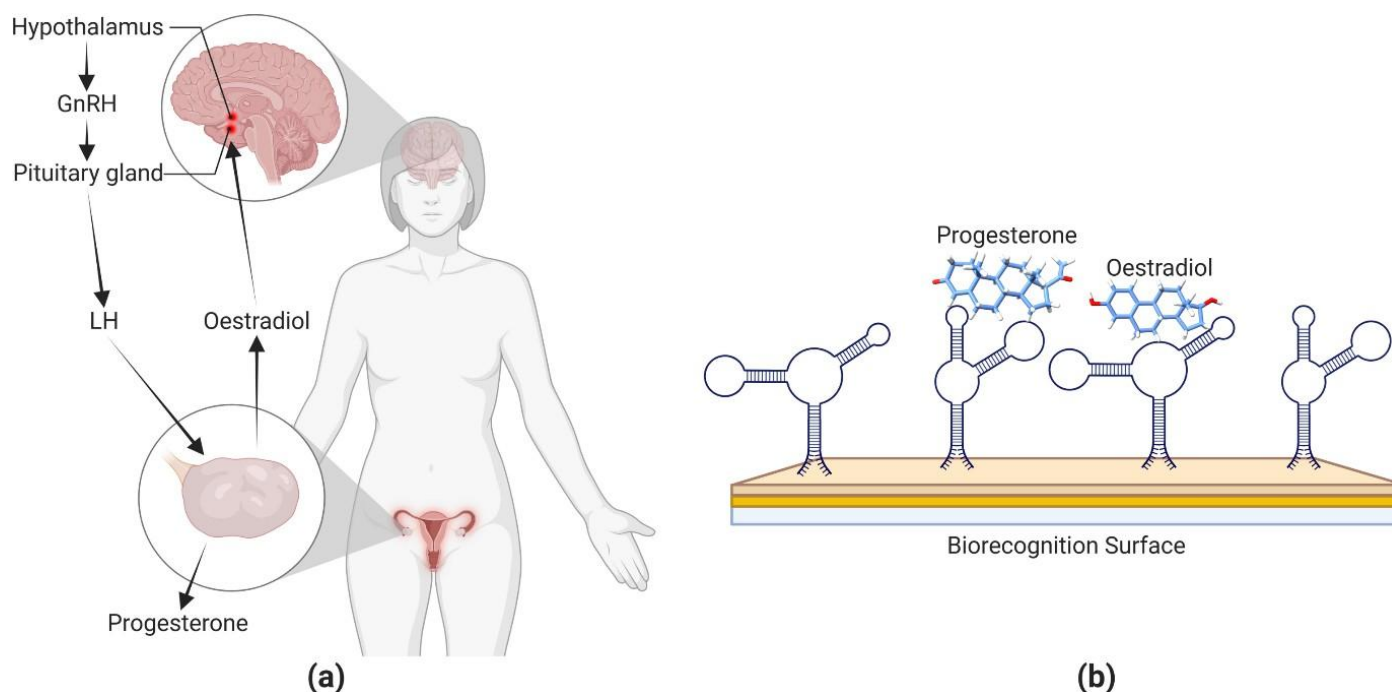


Fig. 1. (a) The hypothalamic-pituitary-gonadal axis: The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the pituitary gland to release luteinizing hormone (LH). LH regulates the production of estradiol and progesterone in the ovaries, influencing reproductive functions. **(b) Biosensing mechanism:** A biorecognition surface functionalized to detect Oestradiol and progesterone molecules utilize specific binding interactions, illustrating their molecular structures and binding configurations.

like LC-MS/MS, which are expensive and require specialized equipment. Techniques like LC-MS/MS and RIA can be time-consuming, potentially delaying clinical decisions. Overcoming these challenges includes selecting appropriate detection methods specific to clinical contexts and ensuring the best practices in assay execution and sample management.

Aptamer is a short, single-stranded DNA or RNA molecule that can bind to specific molecules to target proteins, other small molecules, or cells. (3) Aptamers can be used in many circumstances and applications, including drug delivery systems and therapeutic agents. They can also be utilized in diagnostics, such as biosensors for early disease detection. (4) One limitation that Aptamer might face is that it can be susceptible to degradation by nucleases. They can also be challenged by their in vivo stability, which can affect their effectiveness in therapeutic applications. Current research is focused on developing aptamers with enhanced stability for different applications and improved efficiency in complex biological environments.

Molecular docking is an efficient technique to predict interactions between small molecules and a target molecule. (5) For example, interactions between a drug and a protein can be determined by simulating the binding process. This determines how well one molecule can fit into the active site of a specific protein.

Docking is very often used in drug discovery, biochemistry, and molecular biology. It can help identify potential drug candidates by predicting how well a compound can bind to a target protein. Molecular docking can also be used to study molecular biological systems and design therapeutic agents. (6) One limitation of docking is its accuracy, but some helpful applications include virtual screening and structure-based drug design.

Recently, Liang et al. and Ye et al. developed aptamers that target progesterone and oestradiol concentrations in the brain. (7) we hypothesize that these aptamers have a specific binding region on the aptamer and could be used in hormone detection. In the current research, we performed computational simulations of the aptamers binding to this hormone. we found that all the hormones bind to the large cavity of the aptamers structure, forming hydrophobic interactions. The current research will help us understand the hormone-aptamer binding mechanism and will pave the way for novel methods for hormone monitoring techniques.

2. METHODS

In the current research, we targeted two types of aptamers. The first type binds to oestradiol, and the other binds to proges-

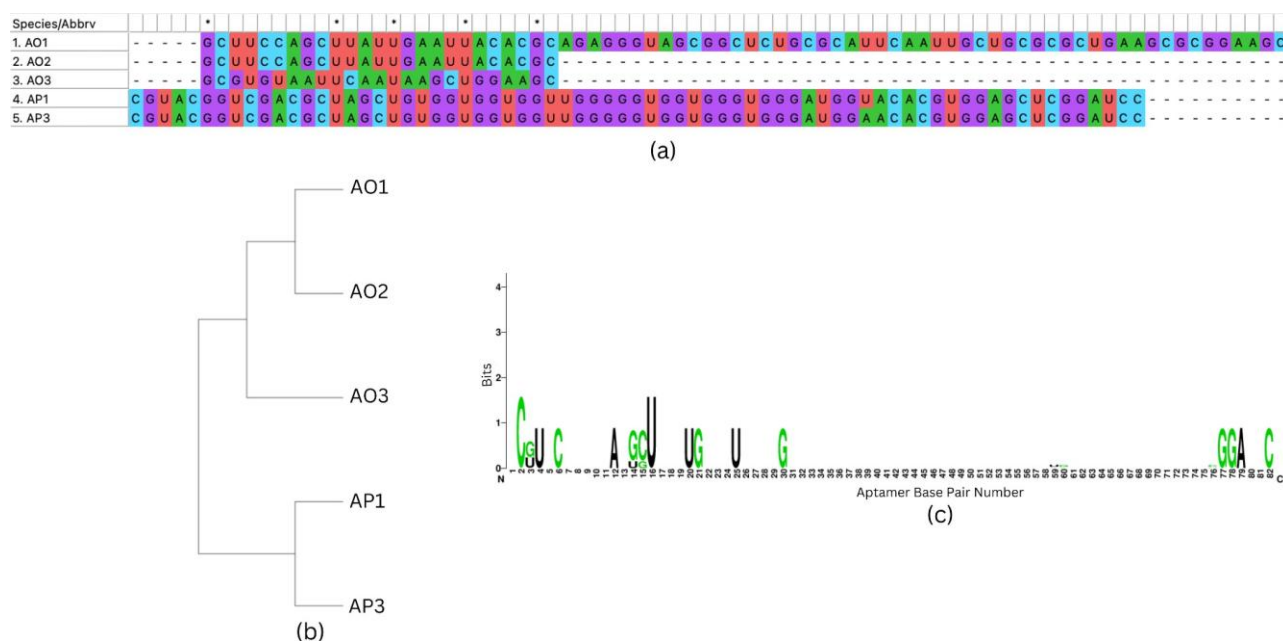


Fig. 2. (a) Demonstrates the sequence alignment of aptamers; (b) The phylogenetic tree based on the sequence alignment of aptamers; and (c) Sequence logo of aptamers shows the aptamer's base pair numbers based on the sequence alignment.

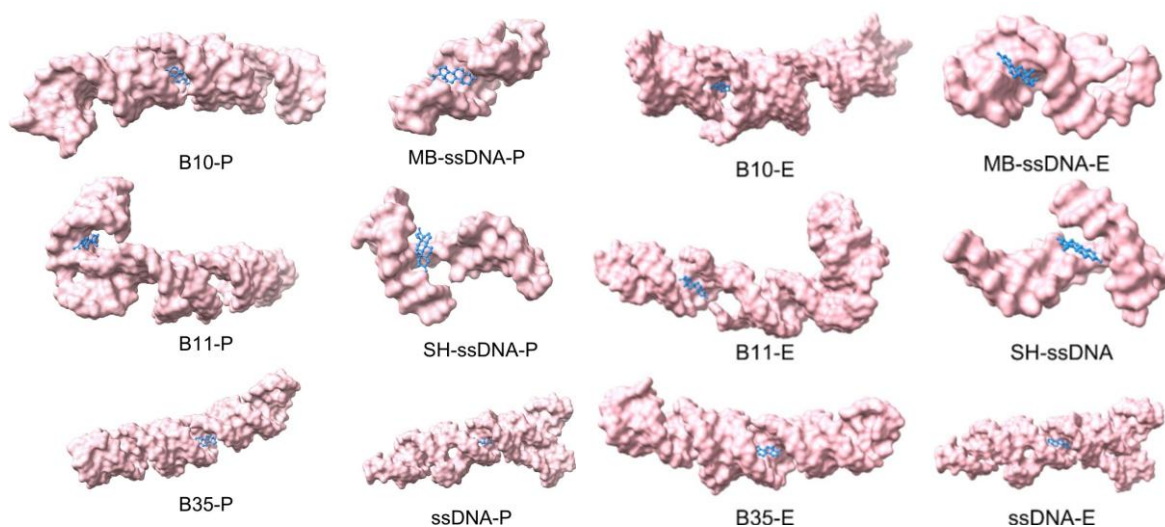


Fig. 3. Aptamer-hormone binding interactions obtained from molecular docking simulations. The aptamer (colored in blue) forms stable complexes with its target hormone (colored in orange), revealing specific binding sites, hydrogen bonding, and complementary structural conformations. These interactions demonstrate the aptamer's potential for selective hormone recognition and targeting.

terone. The aptamer sequences were obtained from recent work done by Ye et al.(7) After receiving the aptamer sequences, they were entered into the Vfold2D site, which can predict RNA 2D structures and give us the 2D version of the aptamer sequences. (8) The generated PNG image was saved and shown in the research paper. The dot and bracket notations are also needed from the site. Then, the copied dot, bracket notations, and the sequence should be entered into the Vfold3D 2.0 site, generating a Template-based RNA 3D structure prediction. The number of clusters on the site must be set to 1, and then it can be submitted for creation. After the job is generated, the molecular docking step can be started on the HDock software. (9)

3. RESULTS

Aptamers are short, single-stranded DNA or RNA molecules folded into specific three-dimensional shapes to bind to a target molecule with high specificity and affinity. (3) The aptamer is the nucleotide sequence that forms the aptamer three-dimensional structure and is used in cancer diagnosis. Sequence similarity analysis compares nucleotide or protein sequences to identify regions of similarity and indicate functional, structural, or evolutionary relationships. The study of the evolutionary relationships among species or genes is phylogenetic analysis and is typically represented as a phylogenetic tree. A phylogenetic tree analyzes similarities and differences in genetic or molecular sequences. A sequence logo is a graphical representation of the conserved regions within sequence alignment. The height of the letters represents the degrees of conservation, showing the frequency of each nucleotide or amino acid at each position.

MOLECULAR DOCKING SIMULATIONS: I used HDock, a molecular docking software, to perform molecular docking simulations. Molecular docking simulations predict interactions between small molecules and a target protein or other biomolecule. They determine how well one molecule can fit into the active site of a specific protein.

Aptamer-hormone docking and confidence score.

Molecular docking scores and confidence scores for various aptamer and ssDNA constructs binding to Estrogen (E) and Progesterone (P). SH-ssDNA-E exhibited the strongest binding to Estrogen with a docking score of -451.62 and a high confidence score of 0.9976, followed by ssDNA-E and B35-E. For Progesterone, B10-P showed the strongest interaction with a docking score of -403.4 and a confidence score of 0.9937. These results suggest that specific modifications to ssDNA and aptamers significantly influence their binding affinities and predictive reliability toward hormone targets.

4. DISCUSSION

The measurement of estrogen and progesterone levels plays a vital role in assessing reproductive health, diagnosing hormonal imbalances, and guiding fertility or hormone replacement therapies. Various analytical techniques are available, each with distinct principles, advantages, and limitations. Immunoassays such as ELISA, RIA, and CLIA are commonly used due to their sensitivity and convenience, though they may suffer from cross-reactivity or require specialized equipment. Advanced methods like LC-MS/MS provide high specificity and accuracy, making

them the gold standard for hormone quantification, albeit at a higher cost and complexity. Non-invasive approaches such as salivary or urinary testing offer user-friendly alternatives, particularly for long-term monitoring. The choice of technique often depends on clinical context, required sensitivity, available resources, and the type of sample collected.

Traditional methods for hormone measurement include immunoassays like ELISA, radioimmunoassay, and mass spectrometry. Even if they are widely used, they have notable drawbacks. Immunoassays are linked to cross-reactivity and lower specificity, which can lead to inaccurate results. Radioimmunoassays that involve radioactive materials can raise safety and disposal concerns. Though highly accurate, mass spectrometry is expensive, requiring complex instrumentation, and demanding skilled operators, which can lead to limited accessibility.

Aptamers allow for precise and non-invasive detection of hormonal imbalance, improving early diagnosis and management of disorders like PCOS and thyroid dysfunction. Aptamer-based sensors help identify hormone-related cancers, like breast and ovarian cancer, by targeting specific hormone biomarkers. For female health monitoring, aptamers can support real-time tracking of hormone levels for fertility assessment, pregnancy monitoring, and menstrual health management. One of the limitations we encountered was looking into the aptamer binding to other hormones and its stability. Aptamers are susceptible to degradation, which could impact their effectiveness.

3: Validation steps, techniques used, and their respective purposes in aptamer development.

The table 3 outlines the key validation steps involved in developing aptamer-based diagnostic tools. It begins with the chemical synthesis of DNA or RNA aptamers to generate the material required for testing. Binding affinity is then assessed using techniques like Surface Plasmon Resonance (SPR), Isothermal Titration Calorimetry (ITC), or Electrophoretic Mobility Shift Assay (EMSA) to confirm aptamer-hormone interactions. Specificity testing through competitive binding ensures the aptamer does not bind to unintended targets. Sensor development involves integrating the aptamer into electrochemical, fluorescent, or lateral flow assay (LFA) platforms to create a functional diagnostic device. Real sample testing using human serum or plasma evaluates clinical applicability. Stability analysis through serum incubation and gel electrophoresis estimates the biological half-life. Structural confirmation via Circular Dichroism (CD) and Nuclear Magnetic Resonance (NMR) validates the aptamer's conformational integrity. Finally, reproducibility is assessed by repeating assays to ensure consistent performance.

CONCLUSION: The research illustrated the capability of aptamers as accurate and non-invasive instruments for identifying hormonal discrepancies, specifically targeting progesterone and oestradiol. Aptamers with excellent specificity and affinity for target hormones were found by computational modeling and docking simulations, facilitating the development of new biosensors for reproductive health and hormone-responsive cancer detection. These findings underscore the benefits of aptamer-based methodologies compared to conventional techniques, including enhanced specificity, less invasiveness, and cost efficiency. Despite continued concerns such as degrading susceptibility and cross-reactivity, aptamer stability, and design improvements are expected to address these issues. This study lays the groundwork for creating aptamer-based diagnostic instruments with potential applications in customized medicine and healthcare.

Table 1. Aptamer-hormone docking and confidence score. Molecular docking scores and confidence scores for various aptamer and ssDNA constructs binding to Estrogen (E) and Progesterone (P). SH-ssDNA-E exhibited the strongest binding to Estrogen with a docking score of -451.62 and a high confidence score of 0.9976, followed by ssDNA-E and B35-E. For Progesterone, B10-P showed the strongest interaction with a docking score of -403.4 and a confidence score of 0.9937. These results suggest that specific modifications to ssDNA and aptamers significantly influence their binding affinities and predictive reliability toward hormone targets.

Estrogen (E)		
Compound	Docking Score	Confidence Score
SH-ssDNA-E	-451.62	0.9976
ssDNA-E	-391.63	0.9921
B35-E	-377.91	0.9896
B10-E	-361.5	0.9857
MD-ssDNA-E	-352.67	0.981
B11-E	-338.82	0.9776
Progesterone (P)		
B10-P	-403.4	0.9937
B35-P	-383.32	0.9907
B11-P	-368.79	0.9876
MB-ssDNA-P	-336.24	0.9765
SH-ssDNA-P	-312.24	0.9656
ssDNA-P	-304.23	0.9624

Table 2. Summary of techniques used for measuring estrogen and progesterone levels.

Technique	Principle	Sample Type	Advantages	Limitations
ELISA (Enzyme-Linked Immunosorbent Assay)	Antibody binds hormone; enzyme reaction causes a color change.	Blood, saliva, urine	Easy to use, cost-effective, good for large sample sizes	Cross-reactivity, less specific than mass spec
RIA (Radioimmunoassay)	Radioactive-labeled hormones compete with natural hormones for antibody sites	Blood	Very sensitive and specific	Radioactive handling required, strict regulations
CLIA (Chemiluminescent Immunoassay)	Similar to ELISA but uses chemiluminescence for detection	Blood, serum	High sensitivity, faster than ELISA	Expensive equipment
HPLC (High-Performance Liquid Chromatography)	Separates hormone from other molecules based on polarity	Blood, serum, urine	High resolution, non-radioactive	Complex, less sensitive than LC-MS
LC-MS/MS (Liquid Chromatography–Tandem Mass Spectrometry)	Identifies hormones by mass after separation	Blood, serum, urine	Gold standard: highly specific and sensitive	Expensive, technically demanding
Salivary Hormone Testing	Measures free (biologically active) hormones using immunoassay or LC-MS	Saliva	Non-invasive, reflects active hormone level	Lower concentrations, affected by contamination
Urinary Metabolite Testing	Measures hormone metabolites in urine	Urine	Provides a longer-term view (24-hour window)	Hormone levels may be influenced by kidney function, hydration

Table 3. Validation steps, techniques used, and their respective purposes in aptamer development.

Validation Step	Technique	Purpose
Aptamer synthesis	DNA/RNA chemical synthesis	Produce material for testing
Binding affinity	SPR, ITC, EMSA	Confirm aptamer-hormone interaction
Specificity testing	Competitive binding	Rule out off-target binding
Sensor development	Electrochemical/fluorescent/LFA	Build a functional diagnostic tool
Real sample testing	Human serum/plasma	Clinical applicability
Stability analysis	Serum incubation, gel	Biological half-life estimation
Structure confirmation	CD, NMR	Validate conformational integrity
Reproducibility	Repeat assays	Consistency in results

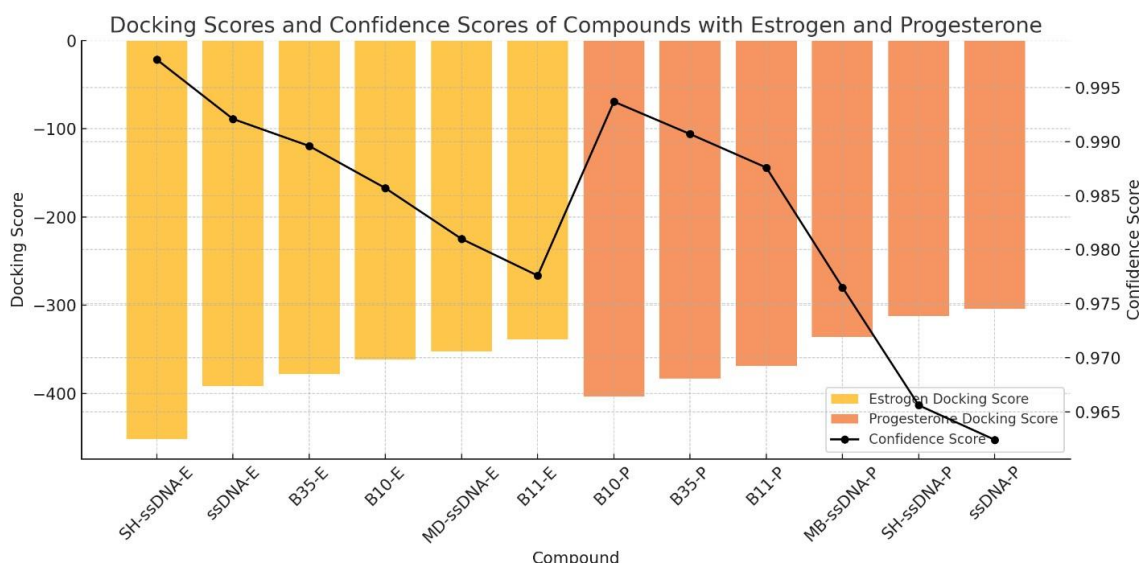


Fig. 4. Docking and confidence scores of various DNA-based compounds with Estrogen and Progesterone receptors. The bar graph displays the docking scores (in kcal/mol) of each compound, indicating binding affinity, with more negative values representing stronger interactions. Compounds targeting Estrogen are shown separately from those targeting Progesterone. The overlaid line plot illustrates the confidence scores of each docking simulation, highlighting the reliability of predicted interactions. SH-ssDNA-E exhibited the strongest binding affinity for Estrogen, while B10-P showed the highest affinity for Progesterone, both with high confidence scores.

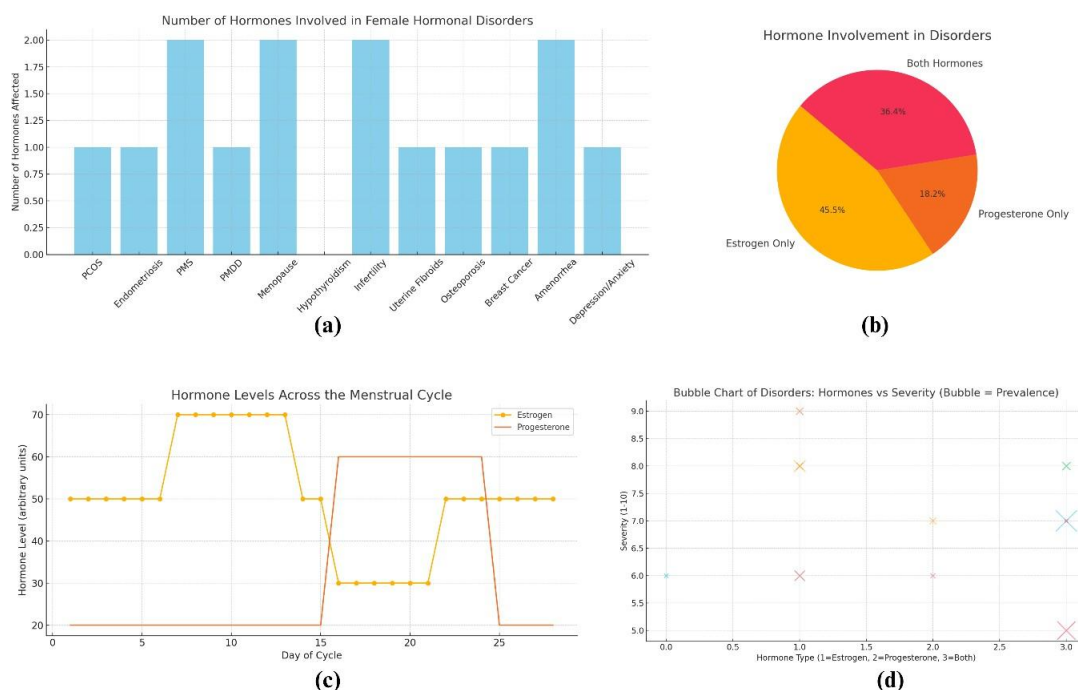


Fig. 5. Overview of hormonal involvement in female hormonal disorders (a) Number of Hormones Involved in Female Hormonal Disorders: A bar chart displaying the number of hormones affected in various female hormonal disorders, including PCOS, PMS, PMDD, infertility, and others. Disorders like PMS, infertility, and breast cancer involve both estrogen and progesterone, while others affect only one hormone. (b) Hormone Involvement in Disorders: A pie chart categorizing disorders based on whether they involve estrogen only (45.5%), progesterone only (18.2%), or both hormones (36.4%). (c) Hormone Levels Across the Menstrual Cycle: A line graph showing the typical fluctuation of estrogen and progesterone levels throughout a 28-day menstrual cycle. Estrogen peaks before ovulation, while progesterone levels rise during the luteal phase. (d) Bubble Chart of Disorders: Hormones vs Severity: A bubble chart mapping disorders based on the type of hormone involvement (estrogen = 1, progesterone = 2, both = 3) and severity (scale 1-10). Bubble size represents disorder prevalence, offering a visual correlation between hormonal basis, impact severity, and frequency.

5. DISCUSSION

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