Radioimmunoassay for measurement of progesterone in cow's milk for studies on Reproductive Efficiency

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Abstract

Antigens (Ag) trigger the immunological reactions in the body. Immune system recognizes and attacks the non-self-external Ag. When immune complexes accumulate in organs, like in some types of vacuities, they can also result in disease. RIA is an extremely sensitive in vitro assay method that is typically used to measure antigen concentrations (e.g., hormone levels in blood) using antibodies. Alkaline phosphatase (AP), glucose oxidase, or horseradish peroxidase (HRP) are among the enzymes employed in ELISAs. RIA measures radioactivity released by bound antibody-antigen complexes. The standard curve, created by plotting antibody-bound radiolabeled antigen against known unlabeled antigen quantities, is used to infer antigen concentrations in patient samples. The RIA measurement of milk progesterone was determined to be reliable and acceptable. As a marker of pregnancy status, progesterone levels in milk samples were determined using RIA. The RIA measurement of milk progesterone in cow was shown to be appropriate and dependable when combined with subsequent rectal palpation. Insufficient luteal phase is linked to insufficient progesterone production. Successful embryo implantation and pregnancy maintenance depend on progesterone.

Keywords: Radioimmunoassay (RIA), Progesterone, Reproductive efficiency, Antibody - Antigen complex

Introduction

A molecule, moiety, foreign particle, or allergen (like, pollen) that can attach to a particular antibody or T-cell receptor is referred as an antigen (Ag). In immunology, an immunological reaction may be triggered by antigens in the body (Schöll et al., 2005). Ag exist in both forms either self or non-self-antigens. "Non-self" external Ag are recognized and attacked by the immune system. Because T cells in the thymus and B cells in the bone marrow are negatively selected, antibodies typically do not respond with self-antigens. Autoimmune disorders occur when antibodies can harm the cells of body by reacting with self-antigens (Chong et al., 2022). Vaccines contain immunogenic Ag, which is administered to activate the immune system's

memory function against the pathogen's Ag. Antigenic specificity refers to the ability of host cells to accurately identify and differentiate between distinct molecular entities of an antigen. The main cause of Ag specificity is the antigen's side-chain conformations, and it is a measureable step. Both B and T cells are responsible for adaptive immunity. One well-known example is the seasonal influenza vaccine. Antigens include proteins, peptides, simple sugars, fat molecules, and polynucleotides. Antigens are present in the functional cells, cancerous cells and microbes (Burton & Dwek, 2006).

Classification of antigens on the basis of sources

Antigens can be classified on the base of their source.

1. Foreign Antigens

Exogenous antigens, entering the body through ingestion, inhalation, or injection, are broken down by the immune system through endocytosis or phagocytosis. T lymphocytes are specific to the MHC complex peptide. Activated T cells release chemicals (cytokines) that stimulate macrophages, antibody-secreting B cells, cytotoxic T lymphocytes (CTL), and other particles. Certain antigens (like, intracellular viruses) are first exogenous before becoming endogenous. After the infected cell is destroyed, intracellular antigens may be released back into the bloodstream (Chong et al., 2022).

2. Endogenous Antigens

Normal cell metabolism or an intracellular bacterial or viral infection can produce endogenous antigens within healthy cells. The fragments are then shown in the complex with MHC class I molecules on the cell surface. The T cells release a variety of poisons that cause the damage of plasma membrane of infected cell. Tolerance eliminates cytotoxic cells, preventing degradation. Endogenous antigens, such as xenogeneic, autologous, idiotypic, or allogenic antigens, can also be present in autoimmune diseases (Kim, 2024).

3. Auto antigens

Auto antigens are self-proteins recognized by the immune system in autoimmune illnesses, causing T cells to attack rather than eliminate them (Factor, 2024).

Neoantigens

Neoantigen is a substance that completely lacks the typical human DNA. Neoantigens are more relevant to tumor suppression than non-mutated self-proteins due to their lack of impact on the quality of the T cell pool accessible for these antigens. It is possible to directly identify and measure neoantigens (Xie et al., 2023).

Viral Antigens

The pool of neoantigens for virus-associated tumors, such as cervical cancer and a subset of head and neck cancers are epitope. These neoantigens arise due to integration and expression of viral genes within host cells that triggers the immune response. The neoantigens in non-viral tumors which results from random somatic mutations (Conarty & Wieland, 2023).

Cancerous Antigens

Cancerous antigens are molecules found on the surface of neoplasms. Cancer-specific antigens are exclusively present in these cells and are typically the consequence of a mutation unique to the cancer. Both tumor cells and healthy cells express tumor-associated immunogens. They are more prevalent antigens. Tumor cells may be destroyed by cytotoxic T lymphocytes that are able to identify these antigens. Tumor-specific DNA changes produce unique peptides, or neo-epitopes, in human cancers that do not have a viral origin (Liu et al., 2017).

Antibody

The immune system uses an antibody (Ab) or immunoglobulin (Ig) that are massive, Y-shaped proteins. These are part of the immunoglobulin superfamily. They recognize and kill disease-causing antigens like bacteria and viruses especially. Antibodies are able to identify almost any size antigen from molecules with a variety of chemical compositions. Every Ab has the ability to identify one or more particular antigens. Ag literally means "Ab generator" since the production of an antibody specific to an antigen is triggered by its presence. The two molecules may precisely bond together because each tip of the "Y" of an antibody has a paratope that binds to a single epitope on an antigen. Through this process, antibodies can successfully "tag" an infection or microorganism for assault (Jordan et al., 2004).

Antibody Structure

An antibody is composed of four polypeptide subunits and has a Y-shaped structure. There are two identical light and heavy chains in each subunit. Each heavy chain's N-terminus joins a light chain to form an antigen-binding domain. The arms of the "Y" shape are made up of two antigen-binding domains. "Fragment antigen-binding" (Fab) domains are the term for them. The heavy chains' C-terminus creates a "fragment crystallization" (Fc) region that facilitates communication with effector cells. Non-covalent and disulfide bonds bind the four polypeptide subunits together. There are three constant areas and one variable region in the antibody's heavy chains. Each antibody differs from the others, yet they all have two identical antigen-binding sites (Schöll et al., 2005).

Production and mechanism of Antibody

When a foreign particle is first encountered by an organism's immune system, macrophages go in and take it down, allowing it to be passed to B cells. Following the presentation of these antigens, B cells start producing a new antibody that has a distinct paratope (the location where the antibody binds to the antigen) that binds with a particular epitope (the location in the antigen where the antibody binds) (Xie et al., 2023). Every B cell lymphocyte produces a distinct antibody directed to a distinct epitope After B cells have finished programming, they produce antibodies that attach to particular diseases and cause our bodies to eradicate them. This is accomplished either by the antibody directly attacking the pathogen (typically in the case of viruses) or by attaching itself to the surface of the pathogen (in the case of bacteria). Antibodies inform the immune system to eradicate the pathogen. When pathogens re-enter the host, they stay there forever, dignified to attack (Kao et al., 2010).

Antigen-Antibody complex formation and functions

A molecule created when several antigens bind to antibodies is known as an immunological complex (Kapingidza et al., 2020). With a particular epitope, antigen and antibody works together as a single unit. Following an antigen-antibody reaction, the immune complexes may undergo opsonization, complement deposition, phagocytosis, or protease processing, among other reactions (Lukácsi et al., 2020) C3b-coated immune complexes may be bound by red blood cells with CR1-receptors on their surface, transported to phagocytes (mostly in the liver and spleen) and then released back into the bloodstream.
The ratio of antigen to antibody determines the size and form of immunological complex. The immunological complex's impact is then determined by this. Fc receptors are membrane-bound receptors found on a large number of innate immune cells that bind the constant regions of antibodies. The intracellular signaling pathway initiates communication between the outside and inside of a cell, the majority of these receptors on innate immunity cells must attach to an immune complex made up of several antibodies since they possess a modest affinity. Additionally, the avidity of binding of these receptors can be increased when several immune complexes unite and bind together. This enables innate immune cells to receive several stimuli simultaneously and keeps them from being activated early (Kanneganti, 2020)
When immune complexes accumulate in organs, like in some types of vasculitis, they can also result in disease. **Type III hypersensitivity** is the immunological complex disorder, caused by this hypersensitivity developing into disease states (Usman & Annamaraju, 2023). **Rheumatoid arthritis** and **scleroderma** are among the autoimmune illnesses that prominently exhibit immune complex deposition. **Lupus** has been linked to immune complexes that accumulate on the surface of immune cells due to an inability to break them down in the lysosome (Uzzaman & Cho, 2012).

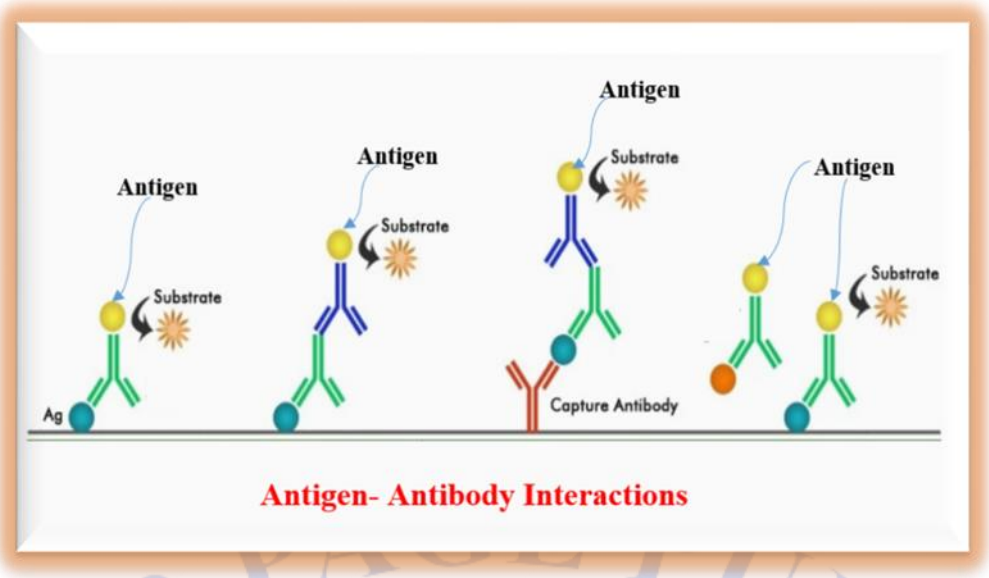

Fig. 1: Representation of Antigen and Antibody Complex

Functions of Antibodies

1. Regulation of antibody production

The control of antibody synthesis can also be influenced by immune complexes. Antigen binding to B-cell receptors (BCRs), which are expressed on the surface of B cells, initiates a signaling cascade that results in activation. B cells express Fc gamma RII b, low affinity receptors exclusive to IgG, allowing IgG immune complexes to bind and prevent cell death. Plasma cells differentiate, expressing gamma II b receptors but no BCR (Daëron, 2024).

2. Regulation and Activation of phagocytes

Immune complexes, especially those made up of IgG, are essential for the activation and regulation of phagocytes such as macrophages and dendritic cells. Immune complexes are more effective than antigen alone. Once more, because many Fc gamma R have a poor affinity for IgG, the signaling cascade of Fc gamma receptors can only be triggered by immune complexes rather than by individual antibodies. The maturation of the vesicles holding the internalized antigen, the activation of DCs and macrophages, and the internalization and processing of the antigen are all significantly altered when immune complexes attach to gamma receptors as opposed to single antibodies (Sun et al., 2021). Different Fc gamma receptors are expressed by various kinds of DCs and macrophages, and these Fc gamma receptors have varying affinities for immune complexes and single antibodies. This makes it possible to accurately adjust the DC's or macrophage's reaction, which in turn adjusts the IgG level. These varied gamma receptors elicit distinct responses in respective DCs or macrophages during distinct signaling pathways. Activation of T cells by DC is initiated by the immune complex (Alon et al., 2021)

3. Removal of opsonized immune complexes

The activation of type I Fc gamma receptors starts a chain of actions that eliminates the IgG-opsonized target. Form I "FcγRs" are another form of IgG constant region receptor that can bind to IgG immune complexes and remove the opsonized complex. Immune complexes bind several type I Fc receptors, which then aggregate on the cell surface to start the ITAM signaling cascade. Although both activating and inhibitory type I receptors can promote phagocytosis, the internalization of IgG-opsonized targets by activating gamma receptors is more effective for response. Immune complexes bind to several type I Fc receptors, which then cluster on the cell surface to initiate the "Immuno-receptor Tyrosine-Based Activation Motif (ITAM)" signaling pathway (Sun et al., 2021) Tyrosine, which is present in the cytoplasmic tail of the molecule and is separated from a leucine or isoleucine by two more amino acids, is the building block of ITAM. FcγRs crosslink to phosphorylate ITAM following the collection of IgG complex. Pro-inflammatory signaling is triggered by the phosphorylation of the ITAM, which

in turn sets off cellular activity, a signaling cascade, and the eventual elimination of the opsonized immune complex (Neves et al., 2020)

Analysis (within the glass)

In vitro refers to a medical investigation or experiment conducted in a lab setting, typically using tissue, cells, or components from an organism to examine disease processes or effects of medication (Ben Mkaddem et al., 2019).

Analysis (within the living)

In vivo refers to a medical test, experiment, or surgery performed on or within a living being, such as a human or laboratory animal (Baig et al., 2024)

Radioimmunoassay

An immunoassay that employs radiolabeled molecules to create immune complexes step-by-step is called a radioimmunoassay (RIA). RIA is an extremely sensitive in vitro assay method that is typically used to measure antigen concentrations (e.g., hormone levels in blood) using antibodies.

Despite requiring specialized equipment, the RIA approach is one of the least expensive ways to do such assessments due to its high sensitivity and specificity (Zarrin et al., 2021).

Labels

A range of labels are used in immunoassays to enable the detection of antigens and antibodies. Usually, labels are conjugated or chemically attached to the target antigen or antibody (Long et al., 2020)

Enzymes

Enzymes are one of the most widely used labels in immunoassays. Enzyme immunoassays (EIAs) are immunoassays that use enzymes. The two most popular varieties are enzyme multiplied immunoassay technique (EMIT) and enzyme-linked immune-sorbent assays (ELISAs). Alkaline phosphatase (AP), glucose oxidase, or horseradish peroxidase (HRP) are among the enzymes employed in ELISAs. Because these enzymes induce a noticeable color shift when specific reagents are present, they frequently make detection possible. These enzymes can occasionally create light or chemo-luminescence when exposed to certain chemicals. In other words, light is emitted as a result of chemical reaction. ELISA comes in sandwich, direct, indirect, and competitive varieties (Guo et al., 2024).

Radioactive Isotopes

A radioimmunoassay (RIA) can be created by adding radioactive isotopes to immunoassay reagents. Conventional techniques make it simple to measure radioactivity released by bound antibody-antigen complexes. RIAs were among the first immunoassays created, however they have become less popular mostly because of the challenges and possible risks associated with handling radioactive (Clarke et al., 2020).

DNA reporters

Combining classical immunoassay methods with real-time quantitative polymerase chain reaction is a more recent approach to immunoassays. A DNA probe is the label utilized in these experiments, which are known as real-time immuno-quantitative PCR (iq-PCR) (Rughetti et al., 2024)

Fluorogenic reporters

Phycoerythrin is one of the examples of such reporters that is utilized in many contemporary immunoassays. One kind of immunoassay that frequently uses these reporters is protein microarrays. Certain labels function by emitting visible light in response to an electric current, a process known as electrochemiluminescence (ECL) (Jullien & Gautier, 2015).

Label-free immunoassays

Although immunoassays typically use some sort of label, certain assay types do not use labels instead they use detection techniques that do not call for labeling or altering the assay's material. One method for identifying the binding of an unlabeled antibody to an antigen is surface plasmon resonance. A further label-less immunoassay that has been demonstrated measures the resistance change on an electrode as antigens attach to it. This label free immunoassays is illustrated in Fig. 2. (Luo et al., 2020).

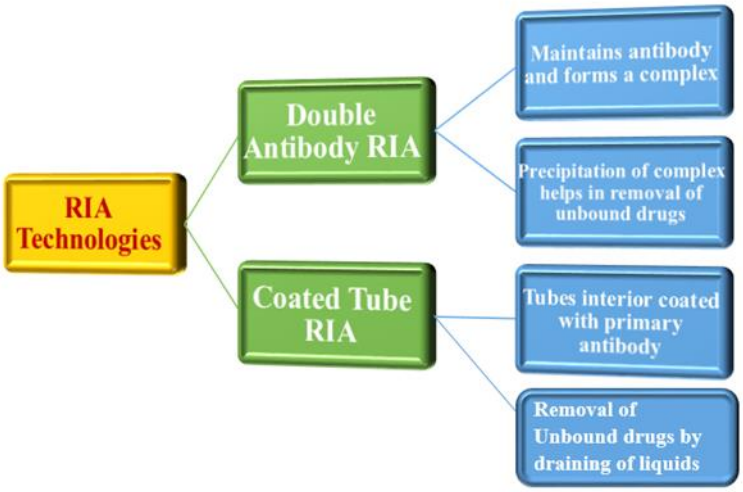

Fig. 2 Representation of Types of RIA

Types of RIA

The counts per minute, which are inversely proportional to the amount of drug in the initial specimen, are determined by evaluating the samples from each RIA method in a gamma counter. Radioimmunoassay requires special handling and disposal of radioactive materials, but it is sensitive and specific (Rughetti et al., 2024).

Radioimmunoassay (RIA) V/S ELISA (Enzyme linked immunosorbent assay)

1. RIA uses radioisotopes to detect antibody-antigen complexes while ELISA uses enzymes to discover them.
2. RIA entails labeling the antigen while ELISA entails labeling the antibody.
3. The RIA assay is more sensitive than the ELISA assay.
4. RIA employs a radioactive label whereas ELISA uses an enzyme label. (Graham et al., 2015)

Fundamentals of RIA (Radioimmunoassay)

Antigens and antibodies binding result in the formation of immunological complex. Certain assay types do not use labels instead they use detection techniques that do not call for labeling or altering the assay's material. Labeled antigens are replaced with unlabeled ones, increasing the concentration of free radiolabeled antigens in the solution when unlabeled antigens bind with antibodies (Randall et al., 2024).

Three principles are combined in this process:

1) Immunological response, such as the binding of an antibody to an antigen

2) Competitive displacement or binding response (It adds precision).

3) Measurement of radio emission which gives sensitivity (Luo et al., 2020).

Immunological response

The body produces specific antibodies against foreign biological material entering the bloodstream through non-oral routes, recognizing the specific chemistry on the surface as an antigen. In response to immunological reaction the body produces antibodies. Antigens or antibodies attach and migrate due to the chemical impact (Edelmann et al., 2021).

Competitive binding or competitive displacement reaction

When two antigens can attach to the same antibody, this phenomenon occurs when the antigen with a higher concentration binds more widely, pushing out the other antigens. Thus, a radiolabeled antigen is permitted to attach to a high-affinity antibody in this experiment. The patient serum then begins to bind to the antibody and displace the labeled antigen when it is introduced to unlabeled antigens (Bozorgchami et al., 2024).

Measurement of radio emission

After incubation, washing removes unbound antigens, and radio emission from the antigen-antibody complex is recorded. It is followed by quantification of gamma rays from the radiolabeled antigen. After being radioactively labeled, the target antigen was linked to its particular antibody having a known concentration. In order to start a competitive response between the preparations labeled antigens and the serum sample's unlabeled antigens. The procedure is explained in Fig. 3 (Janssens et al., 2024).

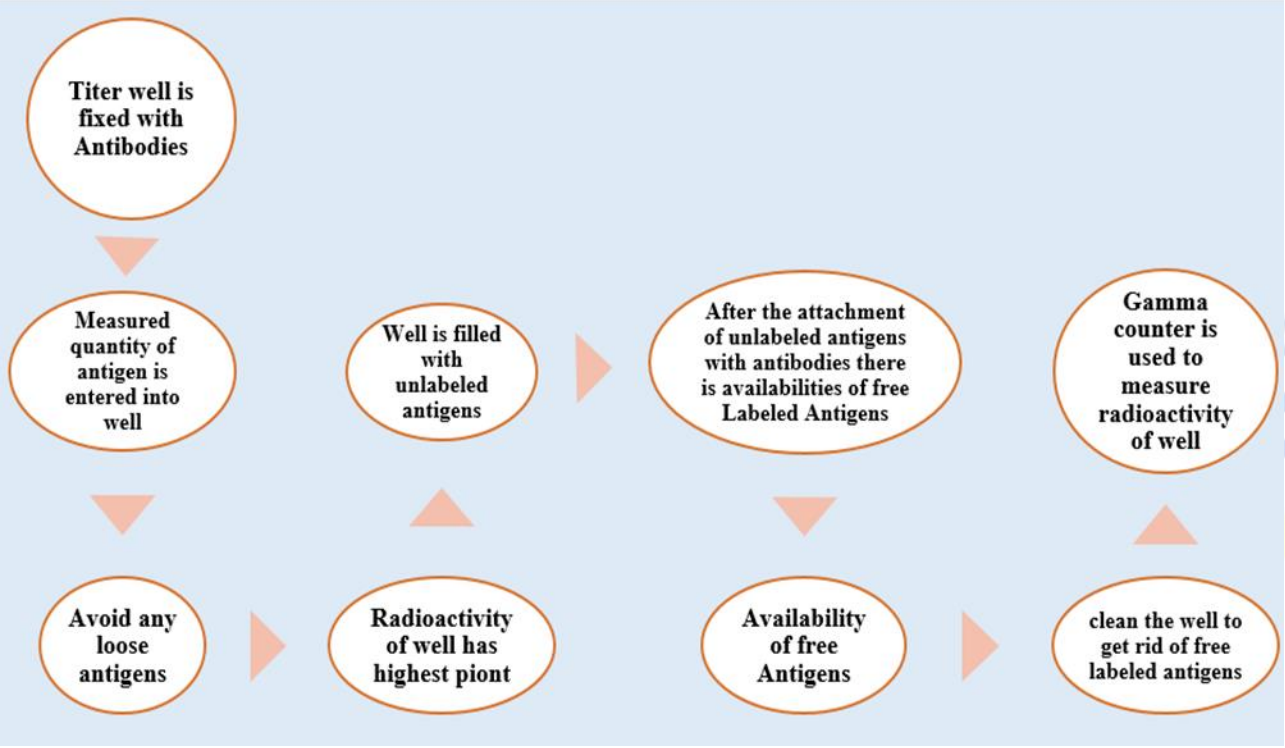

Fig. 3 Formulation of Radioimmunoassay

Table. 1 Quantification of Antigens through Radioimmunoassay (RIA)

Aspect	Details
Method	Radioimmunoassay (RIA)
Process	A sample (e.g., blood serum) is added to particular antibodies. A tagged antigen competes with an unlabeled antigen for antibody binding.
Proportionality	The quantity of released tagged antigen is proportionate to the labeled- to- unlabeled antigen ratio
Binding Curve	Determines antigen concentration in the patient's serum. More unlabeled antigen binds to the antibody and displaces the labeled antigen as concentration rises
Precipitation	Antigen-antibody complexes can be precipitated using c chemicals or crosslinking with another antibody
Measurement	Radioactivity in precipitates is used to calculate antigen concentrations
Sensitivity	RIA has high sensitivity, allowing precise determination of antigen levels in patient samples

Radioimmunoassay (RIA) Procedure

Specific antibodies at a predetermined concentration were used to fix the micro-titer well. The well was filled with a measured number of heated antigens. Extensive cleansing was performed to eliminate any remaining antigens. At that point, the radioactivity of well was at its peak. After that unlabeled antigens were filled in the well. After the unlabeled antigens were attached to the antibody free labeled antigens will remain in the well. For getting rid of the free labeled antigens the well was cleaned again. Gamma-counter was then used to measure the radioactivity of wells The procedure is illustrated in figure 4(Lindland et al., 2024).

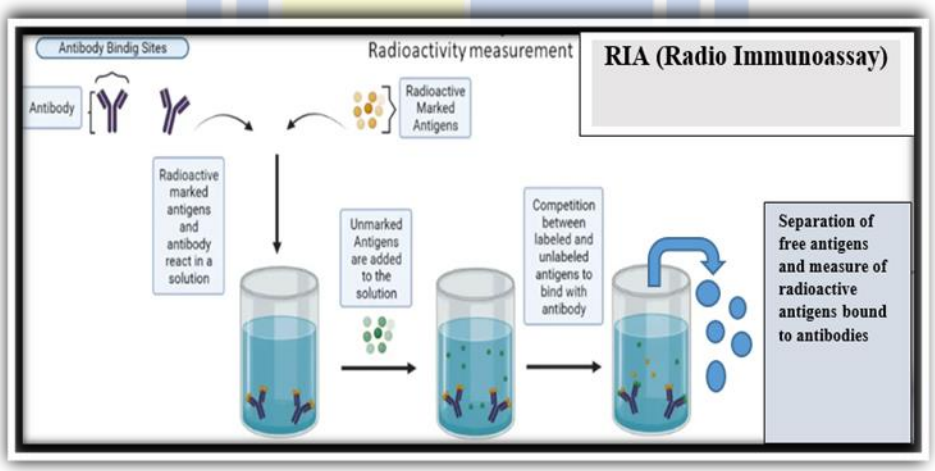

Fig. 4 Radioimmunoassay Protocol

RIA Applications

The detection of peptide hormones was its initial application. Identification of many viral antigens.
Identification of numerous hormones and medications. Hepatitis B surface antigen detection. Detection of toxins that are produced by certain fungi. Early identification of cancer. Endocrinology (hormone measurement). Tumor markers (human chorionic gonadotropin (HCG), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) and prostate specific antigen (PSA). Therapeutic drug monitoring and pharmacology. Contagious illnesses, Autoimmune diseases and allergies, Disorders of metabolism, Health of the reproductive system, Disorders of the Bone and Minerals (Clarke et al., 2020).

Pregnancy diagnosis in dairy cows using milk progesterone determination by RIA

Progesterone levels in milk samples were measured using RIA as a pregnancy status indicator. The most crucial elements influencing the test's possibility were the accuracy and consistency of pregnancy diagnosis. When paired with subsequent rectal palpation, the RIA measurement of milk progesterone was determined to be reliable and acceptable. However, the accuracy may be impacted by elements like nutritional status and estrus detection. Determinations of milk progesterone undoubtedly play a part in diagnosing pregnancy in dairy cows. The measurement of progesterone is explained in Fig. 5 (van der Walt et al., 2007).

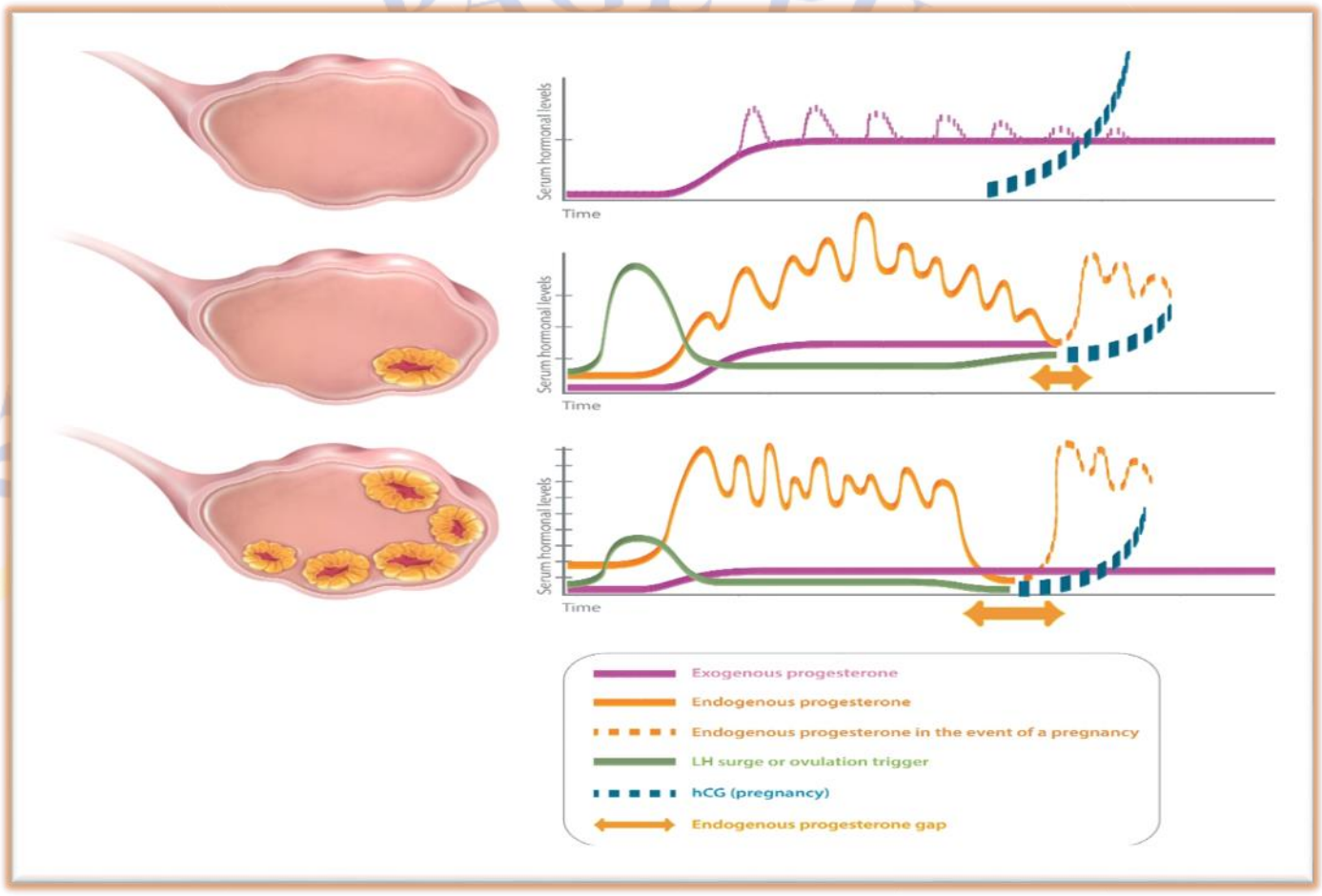

Fig. 5 Effectiveness of RIA Measurement for Pregnancy Detection

Therefore, the presence or lack of a functional CL in the ovary 20 to 24 days after insemination is necessary for the diagnosis of pregnancy. To distinguish between an active CL, a regressing CL, or the lack of a CL, discriminatory levels of milk progesterone must be set.

In order to ascertain (a) the test's accuracy and (b) the milk progesterone's discriminatory limit in determining the presence of an active CL and potential pregnancy, this study assessed the test's ability to diagnose pregnancy in dairy cattle (Xie et al., 2023).

Progesterone: The Key Factor of the Beginning of Life

Pregn-4-ene-3, 20-dione was initially isolated and characterized by George W. Corner and Allen M. Willard, who also acknowledged the significance of this steroid. Progesterone also known as Pregn-4-ene-3, 20-dione, or P4, is the most prevalent hormone generated by the gonads and is derived from the words progestin pro, which means "for," and gest, which means "pregnancy." It is mostly produced by the ovary's corpus luteum, the adrenal cortex, and, in the event of pregnancy, the placenta. The testes and adrenal cortex in men produce progesterone. While its absence results in pregnancy loss, its presence is necessary for embryo implantation (Mesiano, 2022). The prospect of a successful pregnancy is actually decreased by luteal phase abnormalities defined as insufficient luteal phase linked to insufficient progesterone production. Through a variety of non-genomic signaling mechanisms, progesterone also has important extra-reproductive effects. These include neuroprotection, suppression of cholesterol production, immunomodulation, and utero-relaxation (Bulletti et al., 2022).

Successful embryo implantation and pregnancy maintenance depend on progesterone. Many fetal lives are saved by vaginal progesterone medication, which also lowers the risk of premature birth and recurrent miscarriages. Progesterone is much more than just a pregnancy hormone, though. Aldosterone, cortisol, estradiol, testosterone, and other gonadal and non-gonadal hormones all depend on progesterone as a precursor for the synthesis of biologically active steroids. Numerous processes, including the kidney's preservation of sodium, blood pressure regulation, stress response, low blood glucose levels, and the development of secondary sexual traits in both men and women, are attributed to these hormones (Lewis et al., 2024).

Additionally, progesterone has a significant impact on the nervous system. Progesterone's anti-proliferative impact increases a patient's chances of life following severe brain injury, while its neurogenic action is necessary for fetuses' optimal brain development. Progesterone and new progestin serve a variety of vital purposes, such as; endometriosis treatment, luteal phase support, contraception, and the management of dysfunctional uterine hemorrhage. Both the immune response and the prevention and treatment of different types of cancer are significantly influenced by progesterone (Cable & Grider, 2020).

Conclusion

The body's immunological responses are triggered by antigens. The immune system identifies and targets the external, non-self Ag. Disease can also arise when immune complexes build up in organs, as in certain forms of vasculitis. RIA is a very sensitive in vitro assay technique that is commonly used to utilize antibodies to assess antigen concentrations (such as blood hormone levels). The enzymes used in ELISAs include horseradish peroxidase (HRP), glucose oxidase, and alkaline phosphatase (AP). When paired with subsequent rectal palpation, the RIA measurement of milk progesterone in cows was demonstrated to be reliable and suitable. Insufficient synthesis of progesterone is associated with insufficient luteal phase. Progesterone is essential for both successful embryo implantation and pregnancy maintenance.

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