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SUPLEMENTACIÓN DE PRECISIÓN DE SELENIO + VITAMINA E, ÉPOCA DE ANESTRO Y TASA DE IMPLANTACIÓN EMBRIONARIA EN CABRAS

PRECISION SUPPLEMENTATION OF SELENIUM + VITAMIN E, ANESTRUS SEASON, AND EMBRYO IMPLANTATION RATE IN GOATS

TESIS

Que como requisito parcial para obtener el grado de:

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Tesis realizada por **RAQUEL SANTOS SILVA** bajo la supervisión del Comité Asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

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LIST OF ABBREVIATIONS

µm	Micrometer
20 α HSD	20 alpha-hydroxysteroid dehydrogenase
ACBP	Acyl-coenzyme A binding protein
ACTH	Adrenocorticotropic hormone
Ca2+	Calcium ion
CCL8	C-C motif chemokine ligand 8
CL	Comarca Lagunera
CXCL10	C-X-C motif chemokine ligand 10
CYP26B1	Cytochrome P450 family 26 subfamily B member 1
DNA	Deoxyribonucleic Acid
E2	Estradiol
ESR1	Estrogen Receptor Alpha gene
EVs	Extracellular vesicles
FSCN3	Fascin Actin-Bundling Protein 3
FSH	Follicle-Stimulating Hormone
Ges	Superficial glandular epithelium
GnRH	Gonadotropin-Releasing Hormone
GPx	Glutathione Peroxidase
GSH	Glutathione
h	Hour
H_2O_2	Hydrogen peroxide
Hsd3b1	3-beta-hydroxysteroid dehydrogenase/Δ-5-4 isomerase type I
Hsl	Hormone-sensitive lipase
HSP 70	Heat Shock Protein 70
i.e.	That is
IDH	Isocitrate dehydrogenase
IFN-τ	Interferon tau
IL-1	Interleukin 1
IL-10	Interleukin-10
IRF1, IRF2	Interferon Regulatory Factor 1 and 2
ISG 15	Interferon-Stimulated Gene 15
ISGs	Interferon-Stimulated Gene
IU	International units

JAK/STAT	Janus Kinase/Signal Transducer and Activator of Transcription
kDa	Kilo dalton
kg	Kilogram
LC	Corpus luteum
Ldlr	Low-density lipoprotein receptor
LE	Luminal epithelium
LH	Luteinizing hormone
LRP8	Low-density lipoprotein receptor-related protein 8
Mg	Milligram
Mm	millimeter
mRNA	Messenger ribonucleic acid
MRP	Maternal Recognition of Pregnancy
Na+/K+-ATPase	Sodium-potassium pump
NDRG1	N-myc downstream-regulated 1
NK	Natural Killer cells
NOX/NADOH	Nicotinamide Adenine Dinucleotide Phosphate Oxidase
OxyR o OXTR	Oxytocin receptor
P4	Progesterone
PG	Prostaglandin
PGF2α	Prostaglandin F2 alpha
PMNs	Polymorphonuclear cells
Ppm	Parts per million
Rho GDI	RHO protein GDP dissociation inhibitor
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
Se	Selenium
SELENOP	Selenoprotein P
SeVit E	Selenium plus vitamin E
SLC2A	Solute Carrier Family 2
SLC2A1	Glucose Transporter Type 1
SLC2A3	Glucose Transporter Type 3
SLC2A5	Glucose Transporter Type 5
SLC2A8	Glucose Transporter Type 8
SPP1	Secreted Phosphoprotein 1
T3	Triiodothyronine

T4	Thyroxine
TGFβ	Transforming Growth Factor Beta
TH1	T Helper 1 Lymphocyte
TH2	T Helper 2 Lymphocyte
TUBB	Tubulin beta class I
Vit E	Vitamin E
α-TTP	α-tocopherol transfer protein

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RESUMEN GENERAL

SUPLEMENTACIÓN DE PRECISIÓN DE SELENIO + VITAMINA E, ÉPOCA DE ANESTRO, Y TASA DE IMPLANTACIÓN EMBRIONARIA EN CABRAS

Los efectos del cambio climático han provocado una disminución de la disponibilidad de agua en muchas regiones, especialmente en las zonas áridas. Ante este escenario, las cabras se consideran los animales con mayor capacidad de adaptación a estos ambientes. Sin embargo, las drásticas fluctuaciones en la disponibilidad de alimentos y los bajos aportes de nutrientes en las épocas de sequías se reflejan en bajas condiciones corporales y en bajos niveles productivos y reproductivos. El aporte nutricional se considera una opción para incrementar la eficiencia reproductiva. El selenio mejora los atributos reproductivos y ha demostrado buenos resultados en la supervivencia, la implantación y el desarrollo del embrión. La vitamina E protege a los embriones del daño oxidativo y mejora su desarrollo antes de la implantación. El objetivo de este estudio fue evaluar el posible efecto del SeVit E en la tasa de implantación embrionaria en cabras durante la época de anestro. Las variables de respuesta incluyeron la inducción al estro (IE), tasa de ovulación (TO), diámetro del cuerpo lúteo, diámetro de ovario derecho e izquierdo, implantación de embriones (IEMBRI) y tasa de preñez (TPRE) en cabras lecheras cruzadas, adultas y anéstricas (n=32). El grupo suplementado (SeVE; n=16) recibió selenio (5 mg) además de vitamina E (350 UI), mientras que el grupo control (CONT; n=16) recibió solución salina fisiológica. Se indujo a estro utilizando progesterona (20 mg) y gonadotropina coriónica equina (400 UI) y se expuso al efecto macho. Las variables de IE, TO, IEMBRI y TPRE fueron mayores ($p < 0.05$) en el grupo SeVE. La suplementación dirigida mejoró los resultados reproductivos sin afectar el tamaño de la estructura ovárica, lo que sugiere que dicha estrategia mejora la eficiencia reproductiva.

Palabras clave: Eficiencia reproductiva, tasa de preñez, reproducción, embriones.

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GENERAL ABSTRACT

PRECISION SUPPLEMENTATION OF SELENIUM + VITAMIN E, ANESTRUS SEASON AND EMBRYO IMPLANTATION RATE IN GOATS

The effects of climate change have reduced water availability in many regions, especially in arid areas. In this scenario, goats are considered the most adaptable animals to these environments. However, drastic fluctuations in food availability and low nutrient inputs during drought are reflected in poor body condition and reduced production and reproduction. Nutritional supplementation is considered an option to increase reproductive efficiency. Selenium improves reproductive traits and has been shown to enhance embryo survival, implantation, and development. Vitamin E protects embryos from oxidative damage and enhances embryo development before implantation. The objective of this study was to evaluate the possible effect of SeVit E on the rate of embryo implantation in goats during the anestrus period. Response variables included estrus induction (EI), ovulation rate (OR), corpus luteum diameter, left and right ovary diameter, embryo implantation (EMBRYO), and pregnancy rate (PREG) in crossbred, adult, and anestrus dairy goats (n=32). The supplemented group (SeVE; n= 16) received selenium (5 mg) plus vitamin E (350 IU), and the control group (CONT; n=16) received physiological saline solution. Estrus was induced using progesterone (20 mg) and equine chorionic gonadotropin (400 IU) and exposed to the male effect. The variables of EI, OR, EMBRYO, and PREG were higher ($p < 0.05$) in the SeVE group. Targeted supplementation improved reproductive outcomes without altering ovarian structure size, suggesting that this strategy enhances reproductive efficiency.

Keywords: Reproductive efficiency, pregnancy rate, reproduction, embryos

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CHAPTER I

1.1 INTRODUCTION

The effects of climate change have reduced water availability in many regions, particularly in drylands. Projections indicate that this trend will continue to worsen, in part due to population growth, rising energy demand, socio-economic development, and, consequently, food production. In this scenario, goats are considered the animals with the greatest capacity to adapt to these environments by taking advantage of vegetation in disadvantageous conditions (Geldsetzer-Mendoza & Riveros, 2023; Scopinich-Cisternas & Strahsburger, 2019; Nava-Joachin et al., 2023).

Goat production is the activity of raising, handling, and producing goats. This activity is highly valued as it generates environmental services by controlling vegetation, preventing fires, and maintaining natural areas or territories. In addition, it is an important component in the economy and culture of some socially marginalized groups as it helps to improve the quality of life of producers by generating income and high-quality food products with a high nutritional value, which is why it plays an important role in society (Chávez-Espinoza et al., 2021; García-Bonilla et al., 2016; Luna, 2021; Salgado et al., 2023).

The goat population is distributed across many countries worldwide along the fringe between the Tropics of Cancer and Capricorn, where most arid and semi-arid areas are located, as well as in many developing countries, such as India, China, Pakistan, and Nigeria, among others. This behavior makes goats highly distributed (Nava-Joachin et al., 2023; Salgado et al., 2023; Torres, 2015; Valle, 2013). In Mexico, the production of small ruminants plays an important socioeconomic role in addressing poverty in rural and arid areas, due to the income this activity generates and the number of individuals it supports. The national inventory of goats is around 8.7 million heads, placing Mexico in thirteenth place worldwide and second in the Americas after Brazil. In the country, the states with the largest inventory are Puebla, Oaxaca, San Luis Potosí, Coahuila, Guerrero, Zacatecas, Nuevo León, Guanajuato, and Michoacán (Aréchiga et al., 2008; Chávez-Espinoza et al., 2021).

In the Comarca Lagunera (CL), an agroecological region located in the arid north of Mexico (25° LN), goat production is a widely distributed activity that has stood out for the number of animals in this area, with around 400,000 goats. The CL is considered the most important dairy basin in the country due to its contribution to the national goat census, even though goat farming is managed by small producers. Despite the region's high productivity, goat farming in the CL is also affected by drastic fluctuations in food availability and low nutrient inputs during drought, which are reflected in poor body condition and lower productive and reproductive levels. In mammals, such restrictions can affect regions of the hypothalamus that regulate the release and production of pituitary hormones involved in reproductive processes (Bustamante-Andrade et al., 2021; Calderón-Leyva, 2013; Machado-Ramos et al., 2023; Orona et al., 2013; Valle, 2013).

Lack of food is directly and significantly associated with reproductive function at different levels of the hypothalamus-pituitary-gonadal axis in goats. These restrictions are associated with the slow growth of animals and low blood glucose levels. These reactions delay the onset of GnRH pulses (i.e., Gonadotropin-Releasing Hormone), which is why some hormones are involved in the proper development of the central nervous system, and whether or not they initiate puberty and the reproductive stage. These hormones indicate, at the brain level, the animal's metabolic reserves, regulating energy needs and supporting normal reproductive function. Therefore, nutritional intake is considered an option to hormonal treatments, as it increases reproductive and productive efficiency. Prioritizing and correctly managing these elements boosts productivity, improves food security, conserves resources such as water, and reduces environmental impact (Cano, 2016; Meza-Herrera & Tena-Sempere, 2012; Al-Barakeh et al., 2024).

All this makes it necessary to identify new and better food strategies, especially supplementation with energy, proteins, vitamins, and minerals. Selenium is a micronutrient considered necessary for animal supplementation. This mineral plays an antioxidant role and participates as a cofactor in maintaining the integrity of cell membranes and preventing cell death. On the other hand, vitamin E can be supplemented with selenium, which serves as an antioxidant. Because all organisms are constantly under attack by free radicals from normal metabolic activity, they have evolved antioxidant systems to mitigate their negative effects. However, it has been shown that most of the soil in Mexico is deficient in selenium, in addition, the digestibility and absorption of this mineral in ruminants is really low compared to monogastric ruminants, with the absorption percentage being 29 to 35% and 77 to 85% respectively (Carbajal et al., 2012; Domínguez, 2013; Fraire-Cordero et al., 2013; Carbajal et al., 2012).

Selenium modulates immune responses by increasing antibody production and promoting lymphocyte proliferation. So immunity is characterized by a transfer between the mother and the fetus. The nutrition of the fetus during pregnancy is very important to achieve its implantation and development. The success of implantation depends on the existence of a blastocyst with the ability to implant, along with the development of an endometrium that is receptive to the blastocyst and adequate amounts of estrogen and progesterone (Betancourt-Alonso et al., 2006; Pérez, 2016; Bustamante-Andrade et al., 2021).

Ruminants supplemented with selenium and vitamin E show an increase in the number of ovarian structures, as well as an increase in the size of the ovary, in addition to an augmented number of corpus luteum because selenium helps maintain proper function of the ovary. They have higher conception rates, improved sperm transport, greater fertility, and contribute to a reduction in embryonic death in the first month of gestation. In supplemented cows, there is less damage due to pathologies such as metritis, placental retention, and ovarian cysts (Cabrera-Mora et al., 2019; Domínguez, 2013; Galván, 2021).

Previous studies in cows have demonstrated the aforementioned benefits (Ramírez-Bribiesca et al., 2005). In addition, Dahlen et al. (2022) point out that the application of selenium to *in vitro* fertilization in animals such as cattle, dogs, and pigs have had great benefits in embryo development and survival, but few studies have focused on studying the application of this mineral and this vitamin in the measurement of the embryo implantation rate in goats under arid conditions, grazing and in the season of reproductive stillness. That said, the main objective of this study is to evaluate the possible effect of SeVit E on embryo implantation rate compared with the control group in goats at anestrus, under a semi-intensive production system in northern Mexico.

1.2. OBJECTIVE

To evaluate the possible effect of SeVit E on embryo implantation rate compared to the control group in goats at the anestrus season under a semi-intensive production system in northern Mexico.

1.3. HYPOTHESIS

Precision supplementation with Se + Vit E increases the rate of embryo implantation in goats during the anestrus season under a semi-intensive production system in northern-semiarid Mexico.

CHAPTER II

LITERATURE REVIEW

2.1. IMPORTANCE OF REPRODUCTION AND NUTRITION

In all animals, especially in farm animals, reproductive performance is one of the most important factors, but its success depends on genetics, physical environment, management, and nutrition. The latter factor is more directly and immediately linked to reproduction specifically in gametogenesis, follicular development, ovulation, and steroidogenesis (Daghigh et al., 2019). Nutrition is certainly an important factor in achieving successful reproduction. When animals are in a negative energy balance caused by low quality or quantity of food, they tend to have a great influence on the success of pregnancy involving the transcriptome of the endometrium, the blastocyst, and the conceptus since malnutrition can cause an alteration in the endometrial expression of genes progesterone receptors (Amelkina et al., 2025; Susilowati et al., 2024). Poor nutrition disrupts the animal's homeostasis, altering antioxidant systems and leading to excessive free radical production that can bind to various molecules and affect cell membranes, nucleic acids, and proteins (Bešlo et al., 2022). At the same time, reproduction itself can cause stress in the animal, so supplementation with natural antioxidants has been shown to effectively combat oxidative damage (Xiao et al., 2021).

It has been shown that trace elements play a very important role in the body, including the formation of metalloenzymes that participate in other metabolic processes; selenium participates in reproduction and has been shown to be important in the early development of the embryo (Dantas et al., 2019). Selenium is a micronutrient that participates in the antioxidant system, in the metabolism of thyroid hormone, in DNA synthesis, and in the reproductive system of both males and females (Skibniewska & Skibniewski, 2024). Vitamin E is also an antioxidant that participates in reproduction, specifically in the regulation of ovulation, in the development and survival of the embryo and fetus (Daghigh et al., 2019).

2.2. REPRODUCTIVE PHYSIOLOGY AND EMBRYONIC DEVELOPMENT

2.2.1. Embryonic development

Early embryonic development, before the preimplantation period, involves several vital phases: from the unicellular zygote to the multicellular embryo, and to the conceptus phase, where the embryo can be differentiated along with its extraembryonic membranes (Boeta et al., 2023). It all begins when the oocyte is ovulated, it travels through the oviduct until fertilization (Galina, 2021), where the sperm penetrates the oocyte, this act is carried out in the ampule of the oviduct thus forming the zygote, this is a diploid cell where a series of mitotic divisions advances while descending from the oviduct to the placenta (Alvarez-Oxiley, 2022).

In the first mitotic division, known as segmentation, a division occurs that results in two identical daughter cells, which occurs 20 to 30 h after fertilization. These two cells are known as blastomeres. At first, these cells are synchronous, but as the embryo develops, they become asynchronous. In the second division, a four-cell embryo originates, and as cell division progresses, the cells multiply without affecting the volume of the cytoplasm until the stage of 8 to 16 cells, when the transcriptome, that is the complete set of RNA, is activated, and this state is called morula (Boeta et al., 2023; Johnson et al., 2018). In these stages, the embryo's genome must be activated; however, first, the proteins and RNAs that the oocyte inherited allow the correct development and metabolism of the embryo, and it is not until the embryo of eight blastomeres begins to synthesize its own mRNA and its own proteins for its necessary growth and metabolism (Boeta et al., 2023).

The morula initially lacks well-established connections between its internal and external cells (Green et al., 2021). In the case of sheep, it remains in the oviduct for about 3 to 4 days before entering the uterus (Johnson et al., 2018), and as time progresses, its structure begins to compact due to the establishment of cell junctions. In the central cells, called GAP, and in the end cells, called tight junctions, they are grouped into peripheral and central blastomeres (Boeta et al., 2023).

These peripheral cells release sodium into the intercellular spaces, creating an osmotic pressure that draws water into the embryo until they begin to separate and develop a more marked differentiation between the peripheral and central cells. The cavity that fills with fluid is called a blastocele, and the embryo is called a blastocyst; both occur around day 6 postfertilization. The entire osmotic gradient that allows the formation of the blastocele is established by the Na+/K+-ATPase, also called the sodium pump, which is found in the basement membrane of the outer cells (Boeta et al., 2023; Johnson et al., 2018; Green et al., 2021).

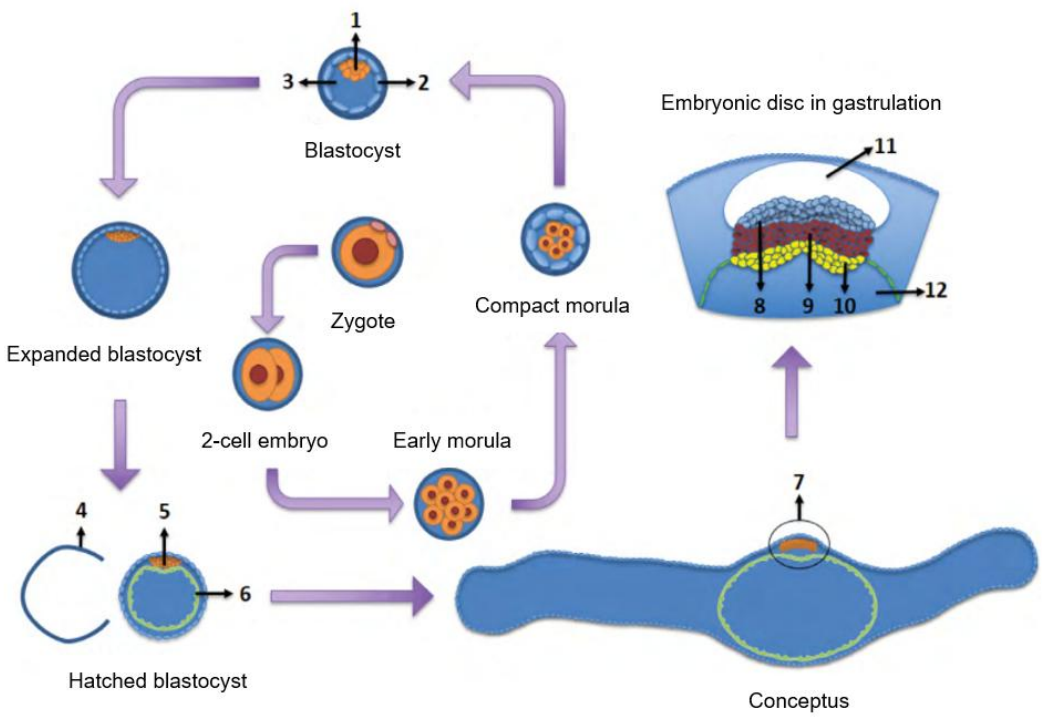
The trophoblast, also known as the trophectoderm, originates from the cells found in the periphery, and it will eventually become the embryo-fetus (Johnson et al., 2018). Inside the inner cells, the embryoblast, which will become the fetus, is established, and once development continues, the embryoblast re-differentiates into primitive endoderm and epiblast. As the development of the epiblast progresses, the gastrulation process takes place, which consists of the origin of the three germ layers called endoderm, mesoderm, and primitive ectoderm, which, together with the trophectoderm or placental chorion, are called the conceptus (Boeta et al., 2023; Johnson et al., 2018).

As the blastocyst continues to grow, the blastocele fluid also increases, which increases pressure on the zona pellucida that covers the embryo. With the joint effect of the proteases that the embryo produces, the moment is reached when the zona pellucida ruptures and hatches (Boeta et al., 2023). This occurs around day 8 and 9, in cattle it can be up to day 10, where its diameter can reach a size of up to 200 μm (0.2 mm) and an approximate of 300 cells and then grow up to 400-900 μm (0.4-0.9 mm) with around 400 to 900 cells, this happens before undergoing a morphological transition called elongation where the conceptus acquires a spherical shape and then ovoid (Johnson et al., 2018; Green et al., 2021).

During elongation, the rapid proliferation of the blastocyst results in a long, filamentous embryo that grows slowly from 1 mm to 8 mm between days 12 and 14 of gestation (Amelkina et al., 2025; Green et al., 2021). In turn, the extraembryonic membranes of ruminants continue to form, and by day 16, an embryonic disc appears around the conceptus, then descends, and the trophoblast layer folds around. This closure of the trophoblast fold over the embryonic disc establishes the amniotic sac and creates a cavity where fluid will accumulate within the amniotic cavity (Green et al., 2021).

By days 15 to 18, the conceptus continues to grow and elongate, reaching up to 200 mm. All this occurs before coming into direct contact with the endometrium (Boeta et al., 2023) located in the uterine horn, ipsilateral to the corpus luteum. Before implantation, the embryo is in free life, and its duration can range from 2 to 3 weeks after fertilization in ruminants. However, in cattle, a very weak attachment to the uterine epithelium is established during elongation; approximately from day 23, the embryonic disc begins to form a connection with the endometrium (Alvarez-Oxiley, 2022; Green et al., 2021). There are also trophoblast papillae, which have been thought to develop as part of the anchoring of the peri-insertion conceptus to the surface of the uterus during conceptus elongation (Green et al., 2021).

The elongation of the conceptus in ruminants and pigs requires great morphological remodeling and cell migration, which is achieved through the supply of ATP, glucose, hexoses, and fructoses, which increase once the elongated conceptus encounters the uterine lumen. Glucose, together with fructose, is transported across cell membranes by the action of the group of proteins called solute transporter family 2A (SLC2A, SLC2A1, SLC2A3, SLC2A5, and SLC2A8). The metabolism of hexoses is carried out by the Krebs cycle and oxidative phosphorylation (Johnson et al., 2025). This metabolic process involves high nutrient and oxygen consumption, which produces a hypoxic intrauterine environment; therefore, the conceptus is under metabolic stress, possibly leading to a shift from oxidative to glycolytic metabolism during elongation. Therefore, it can perform active aerobic glycolysis, a process similar to that observed in tumors and in activated immune cells found in hypoxic environments (Johnson et al., 2025).



Source: Boeta *et al*., 2023

Figure 1. Stages of embryonic development

In sheep, during the elongation stage, the conceptus use the metabolism of one carbon and the pentose phosphate pathway where glucose and fructose contribute carbons to the latter mentioned pathway; then, glutamine comes into action, this non-essential amino acid is certainly required for normal fetal development (Johnson et al., 2025; Cruzat et al., 2018; McIntyre et al., 2020). Once its concentration increases in the uterine lumen during the preimplantation period and in trophoblast cells, the endoderm of the conceptus synthesizes this amino acid, and then the trophoblast of the conceptus consumes it during its elongation, as well as other proteins are synthesized to achieve this event (Johnson et al., 2025).

2.2.2. Immunology of pregnancy

In ruminants, it is estimated that pregnancy failures amount to up to 10% due to fertilization failures and 50% is lost due to embryo mortality, so the important events in pregnancy that are key elements to prevent pregnancy losses must be studied. During pregnancy, both the maternal and the conceptus immune systems play crucial roles (Constantino et al., 2021). Immunity refers to the interactions between the body's defense mechanisms against foreign agents. These mechanisms that participate in the body's defense include natural immunity (innate or non-specific immunity) and acquired, adaptive, or specific immunity (Salazar & Ávila, 2014).

The first line of defense consists of physicochemical barriers; if these are overcome, the innate immune response begins. This line of action carries out phagocytosis, inflammation, the acute-phase response, the complement system, and NK cells. In acquired immunity, T and B lymphocytes play an important role (Salazar & Ávila, 2014). The immune response is activated by lymphocytes, which are cells capable of recognizing alloantigens and are divided into three groups: T cells, NK cells, and B cells. TH lymphocytes are classified according to the cytokines they secrete; they exhibit distinct patterns and therefore perform distinct functions (Rodríguez-Purata & Cervantes-Bravo, 2020).

Two important immunological events prevent rejection of the conceptus during the early stages of gestation. First, in mating, the maternal reproductive tract comes into contact with semen, and the expression of immune modulators, including interleukins, is promoted. The second event is when trophoblast cells come into contact with the maternal endometrium (Constantino et al., 2021). Therefore, for the conceptus to be successfully implanted, it must overcome two important challenges: preventing the mother's immune cells from attacking and inducing remodeling of the endometrium and angiogenesis for the formation of the placenta (Ott et al., 2014). This is achieved by inducing redistribution of the mother's immune cells and altering their function to ensure the maternal response to the conceptus's alloantigen. The conceptus induces alterations in immune function and extends these effects to peripheral blood immune cells during the early stages of maternal recognition of pregnancy (Ott *et al*., 2014).

Since the 1950s, Medawar proposed three mechanisms by which the allelogenic conceptus evades the mother's immune system: 1) the conceptus is inert and does not manifest histocompatibility antigens, 2) the mother's immunosuppression, and 3) the placenta creates a barrier between the conceptus and the maternal immune cells (Constantino et al., 2021; Ott et al., 2014). Gestation is characterized by a shift in the ratio of TH2 to TH1 cytokines, with TH2 dominant at this stage. The prevalence of this cytokine in early pregnancy is due to increased progesterone levels. The embryo also participates in the TH2 domain since it secretes IL-10 (Interleukin 10) and TGFβ (Transforming Growth Factor Beta) (Rodríguez-Purata & Cervantes-Bravo, 2020). The oviduct, like the placenta, contains large numbers of immune cells that are resistant to immune suppression and secretes a wide variety of cytokines. Before implantation, the embryo is influenced by cytokines and growth factors from the oviductal luminal compartment and by uterine epithelial cells. Embryos have cytokine receptors from conception to implantation (Galina, 2021).

After implantation, the blastocyst is surrounded by trophectoderm, which develops into two layers: the primary syncytiotrophoblast and the extravillous trophoblast. The latter is the one that will penetrate the uterus to join the bloodstream. Because of this, the extravillous trophoblast is the only part of the embryo that will be in direct contact with the mother's immune system by invading the decidua and myometrium. The other cells of the fetus are completely isolated from the mother's immune system by the placental trophoblastic barrier (Rodríguez-Purata & Cervantes-Bravo, 2020).

2.2.3. Maternal recognition of pregnancy and interferon-tau

In eutherian animals, i.e., mammals with a chorioallantoic placenta, there is a biochemical dialogue between the conceptus and the mother to establish gestation (Ott et al., 2014). This is called maternal recognition of pregnancy (i.e., MRP) and is defined as the physiological process in which the conceptus signals the mother of its existence. In turn, it extends the life and functioning of the corpus luteum (LC) (Kowalczyk et al., 2021).

For maternal-fetal receptivity to be established, the actions of two maternal steroid hormones, estradiol (E2) and progesterone (P4) (Amelkina et al., 2025), are necessary; the latter is essential for maintaining pregnancy (Bazer et al., 2015). During pregnancy, prostaglandins (PG) F2 α are responsible for performing luteolysis; on the other hand, prostaglandins E2 contribute to the establishment of pregnancy because they protect the corpus luteum and therefore the production of progesterone. These PG are produced by endometrial cells (Elizondo, 2021). In the pre-implantation period, prolactin and placental lactogen are secreted; in turn, there is a change in the shape of the trophoblast from simple cubic epithelium to biseriate cubic epithelium. These changes are programmed by P4, and early increases in this are associated with greater development and elongation of the conceptus. In the same way, progesterone drives the action of the transcriptome, proteome, lipidome, and metabolome of the uterus, which participates in the growth and elongation of the conceptus, as well as in the development and survival of the placenta in later stages (Galina, 2021; Green et al., 2021).

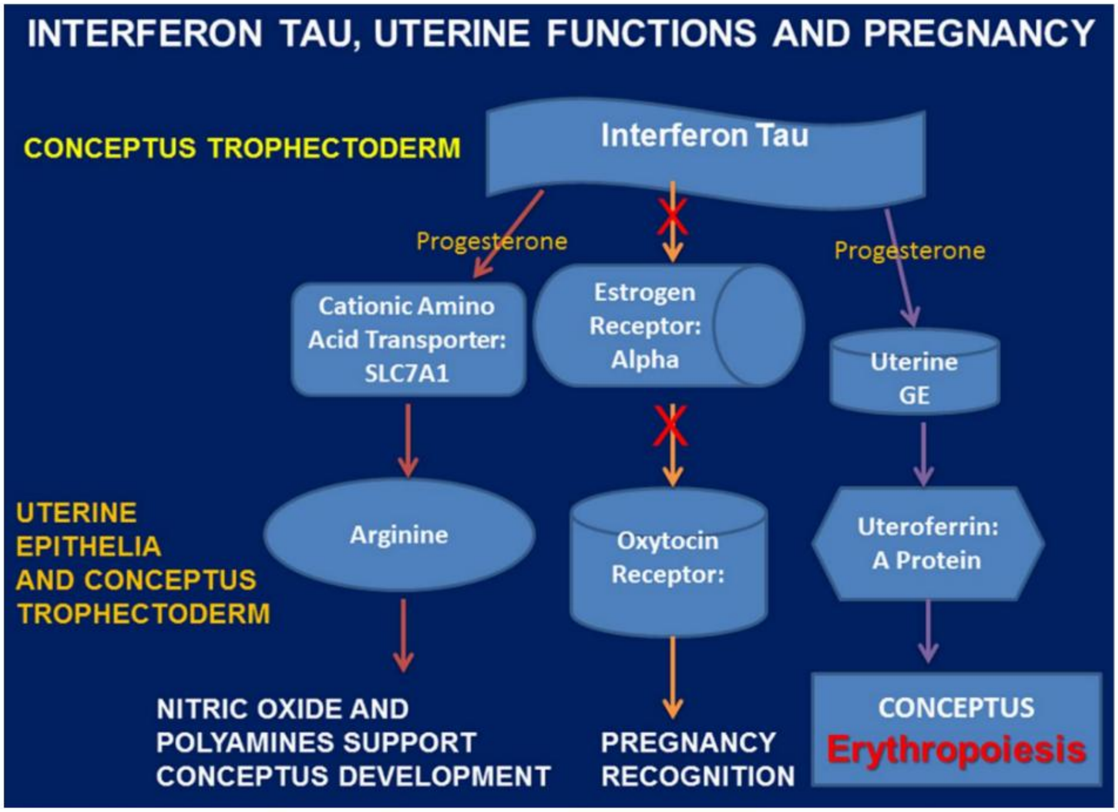
The MRP process is driven by various molecular signals. In some species, such as primates, specific signals by LC are generated by chorionic gonadotropin; in pigs, by estrogens; in rodents, by lactogenic hormones; and in ruminants, by interferon-tau (Bazer et al., 2015). In ruminants, the elongated, filamentous shape change of the trophoblast is used to produce interferon-tau (IFN-τ), a glycoprotein with a molecular weight of 19 to 24 kDa depending on its glycosylation state; according to species, its structure can differ (Bazer et al., 2015). IFN-τ is produced and secreted by the conceptus from day 7 of gestation and by day 16 the conceptus is competent enough to secrete the adequate amount of IFN-τ to trigger maternal recognition of pregnancy and implantation as it acts on various cellular components of the endometrium (Amelkina et al., 2025) however, it is not until day 20 that it reaches its maximum secretion, which depends largely on the amount of progesterone produced by the corpus luteum (Constantino et al., 2021; Fraire, 2015). IFN-τ has a paracrine action on the luminal and glandular epithelium of the endometrium and stroma (Torres, 2013), its secretion plays an important role in the inhibition of luteolysis, to suppress the effects of ESR1 (Estrogen Receptor) in uterine epithelial cells avoiding the regulation of ESR1-dependent

OxyR pulses, as well as those of PGF2 α, ensuring the production of progesterone by the corpus luteum in a constant way to prevent the development of the luteolytic mechanism of the endometrium that requires the pulsatile release of PGF that is induced by oxytocin (Constantino et al., 2021; Elizondo, 2021; Wiltbank et al., 2023; Bazer et al., 2015).

The actions of IFN-τ go beyond inhibiting the expression of the OxyR and ESR1 pulses by preventing the release of prostaglandin synthase 2 and blocking the lysis of LC (Amelkina et al., 2025), as it also induces the expression of genes in the endometrium, one of which is ISG15 through the binding to the interferon alpha 1 or 2 receptor (IRF1 and IRF2) of its activation and subsequent signaling JAK/STAT, which participate in the receptivity of the uterus for implantation and prevention of immunological rejection of conception (Amelkina et al., 2025; Constantino et al., 2021). In sheep and pigs, interferon-tau and estrogens promote the expression of the regulatory factor interferon 2 only in the luminal epithelium (LE) of the uterus and in the superficial glandular epithelium (GEs), silencing the expression of classic genes stimulated by interferon and in turn facilitating the expression of novel genes that allow the development of the conceptus and in turn, increasing the transport and secretion of nutrients such as glucose and amino acids (Bazer et al., 2015).

Although the role of some classical ISGs has not yet been fully discovered, it is known that genes such as CCL8 and CXCL10 participate in the elongation of the conceptus, adhesion and interaction between the embryo and the endometrium, in turn interferon tau stimulates the expression of non-classical ISGs that participate in elongation or implantation, some such as SPP1 are part of the union of the conceptus, some others modify the uterine luminal fluid as well as the elongation of the conceptus (Amelkina et al., 2025).

Another function of the IFN-τ within the uterus is to increase the transport and secretion of growth factors, facilitate the movement of proteins, and support the proper growth and development of the conceptus (Bazer et al., 2015). In sheep, proteins produced by the embryo (days 14-15) have been shown to prolong the corpus luteum lifespan up to day 25. The developing embryo at day 13-21 in sheep and 16-25 in cows secretes interferon-tau (IFN-τ) inside the uterus (Wiltbank et al., 2023).



Source: Bazer et al., 2015.

Figure 2. Interferon-tau pathways.

Interferon-tau and progesterone induce progestamedines that act on the uterine glandular epithelium in order to secrete uteroferrin that promotes the stimulation of erythropoiesis, in the same way, IFN-τ and P4 increase the transport of the rest of arginine usable for the trophectoderm conceptus and for the uterine epithelium that will be used for the metabolism of nitric oxide and polyamines necessary for the development and growth of the conceptus (Bazer et al., 2015), as they stimulate histotroph secretion and nutrient transporter expression. Another role of IFN-τ is the immunological recruitment of immune cells, the inhibition of lymphocyte proliferation, and the stimulation of NK cells, possibly to promote an immunotolerant environment for embryo development (Fiorenza et al., 2021).

Interferon tau modulates polymorphonuclear cells (PMNs) by inducing immunoglobulins and genes that are related to the immune response. PMNs are the first line of defense for organisms against an aggressor agent. Although at first, they were considered villains, they realized that they participate in specialized processes such as pregnancy. Pregnancy-related factors can modify the phenotype of polymorphonuclear cells, depending on the situation, to support the development of the embryo or fetus. The production of IFN-т ends with the start of implantation (Fiorenza et al., 2021). It should be known that the conceptus, apart from producing IFN-т, also produces proteins that participate in the endometrial transcriptome, as well as in the extracellular vesicles (EVs) of some ruminants (Amelkina et al., 2025). These extracellular vesicles are produced by reproductive cells, including follicular, oviductal, and endometrial cells, as well as by embryos, which transfer information to other cells and can cause biological effects such as increased cell proliferation, migration, and adhesion (Nakamura et al., 2020). Evidence indicates that extracellular vesicles derived from the conceptus and endometrium interact with ovarian progesterone, IFN-т, and PG, all of which are necessary for implantation. It is known that a high P4 production is directly associated with a high number of extracellular vesicles. In turn, it was found that the EVs of the uterus contained IFN-т and expressed some genes, but extracellular vesicles also exerted biological effects independent of interferon tau (Nakamura et al., 2020).

2.2.4. Embryo implantation

Embryo implantation is the result of several tissue processes starting with the fixation of the blastocyst in the uterus and ending with the complete formation of a placenta (Betancourt-Alonso et al., 2006). Inside the uterus is the endometrium, which is composed of various cell types, including epithelial, stromal, immune, and endothelial (circulatory system) cells (Galina, 2021). The implantation process can vary across species, so three categories are established based on events that occur during the interaction between the embryo and uterine cells. These are the types of central, eccentric, and interstitial implantation. In ruminant animals it is of the centric type because the blastocyst begins to grow and instead of implanting in the endometrial wall it fuses with the apical membrane of the lumen epithelia without being invaded and the gestation develops in the lumen of the uterine body, this type of implantation is also called non-invasive (Jamwal et al., 2024; Amelkina et al., 2025; Johnson et al., 2025). This occurs because there is a morphological difference in the implantation of ruminants, since binucleated trophoblastic cells are formed in the embryo that manage to migrate and join the uterine epithelium to create a tissue between the embryo and the mother formed by individual syncytia, due to this, implantation and placentogenesis is non-invasive, and in turn maintains the maternal barrier (Amelkina et al., 2025). The success of implantation depends on the existence of a blastocyst with the ability to implant itself and, at the same time, on the development of an endometrium that is receptive (Betancourt-Alonso et al., 2006). At this stage the conceptus faces several challenges during the implantation period (Ott et al., 2014).

As already mentioned, the endometrium is key during gestation, as it supports implantation and nourishes the embryo to ensure a successful delivery; however, the endometrium is reluctant to implant the embryo throughout the estrous cycle, except for a short period (Galina, 2021). In this short period, the endometrial tissue is receptive, expressing the molecules necessary for implantation and, subsequently, for the blastocyst's invasion. This period is also known as the implantation window (Jamwal et al., 2024). This process is governed by endocrine, paracrine, and autocrine modulating factors of maternal and embryonic origin (Gutiérrez & Gutiérrez, 2019). During this time, the luminal epithelial cells show cytoplasmic protrusions with characteristics similar to inflammation: the stroma is edematous and highly vascularized, and large glands with abundant secretions. In other words, during this implantation window, processes similar to those of cell injury can be observed, suggesting that the endometrium shows weakened tissue, and the embryo takes advantage of this state to implant (Galina, 2021).

This process occurs when the blastocyst in the wild begins to penetrate the mucosa of the uterus. Implantation involves a series of stages characterized by the degree of contact between the embryonic and maternal tissue. The three stages of implantation are: apposition, adhesion or fixation, and penetration or decidualization (Alvarez-Oxiley, 2022). These actions depend on the proper function of the ovary, particularly the corpus luteum, which produces progesterone; low progesterone concentrations significantly compromise the process (Bustamante-Andrade et al., 2021). In addition, estrogens play a significant role in embryo implantation and development (Wiltbank et al., 2023). In the same way, enzymatic functions and glycoproteins are efficiently involved in keeping the blastocyst attached to the endometrium, and due to the hydrophilic nature of glycoproteins, they can perform this function in an aqueous environment where they are also involved in cell-to-cell identification and binding with other molecules (Tsantsaridou et al., 2020).

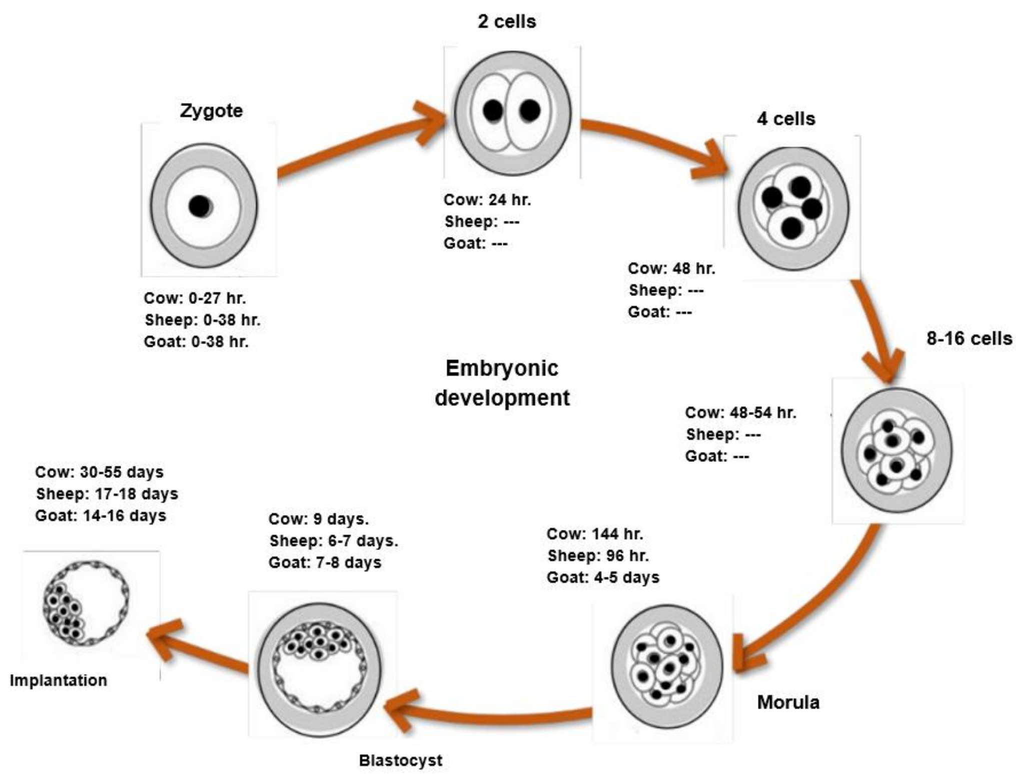
According to Jamwal et al. (2024), little is known about the mechanisms regulating implantation in ruminants; however, proteomics has been used to elucidate these mechanisms. With the use of approaches based on quantitative proteomics and laser desorption/ionization mass spectrometry, it has been found that there are four proteins in endometrial tissues of pregnant cows: the Rho beta GDP dissociation inhibitor (Rho GDI), 20 α-hydroxysteroid dehydrogenase (20 α HSD), isocitrate dehydrogenase (IDH) and acyl-CoA-binding protein (ACBP) that were not found in non-pregnant cows. which suggests that their abundance is related to the implantation process in ruminant animals. These proteins are involved in other processes, such as the Krebs cycle, and are part of antioxidant activity and some biosynthetic pathways (Jamwal et al., 2024). Once the embryo is fixed, signaling between the blastocyst and the uterus begins, initiating placenta formation (Betancourt-Alonso et al., 2006). For blastocyst development in utero, chemotactic guidance, blastocyst adhesion to the uterine epithelial surface, and decidualization are important (Tsantsaridou et al., 2020).

Decidual cells produce locally active molecules, which are vital for directing and limiting trophoblast invasion and leukocyte transport to the decidua or endometrium, and this will have to occur until the placenta is established. Stromal cells have also been found to secrete cytokines that primarily contribute to decidualization, subsequently secreting chemokines that attract leukocytes involved in trophoblast migration (Tsantsaridou et al., 2020). For embryo implantation to occur, there are some factors such as the coordination of maternal and embryonic processes, the transformations of the uterus, fetal development and protection against predators, the exchange of nutrients, the communication between the embryo and the mother, the changes and adaptations of the immune system and the micro-RNA system are directly related to implantation (Tsantsaridou et al., 2020). If there is no synchrony between embryonic development and uterine preparation, implantation may fail, resulting in embryo resorption or miscarriage (Galina, 2021). In goats, the duration of the blastocyst stage is 6 to 7 days, entry into the uterus is 2 to 4 days, and the onset of implantation is 15 to 16 days with an epitheliolionic placenta (Betancourt-Alonso et al., 2006).

2.2.5. Placental formation and fetal nutrition

Nutrition during pregnancy plays an important role in fetal and placental development, as the fetus is nourished by two sources: the histotroph during the preimplantation period and the hemotroph during the embryonic implantation stage. These substances are essential for fetal development and growth; they are provided by the uterine endometrium or directly from the mother's blood (Galina, 2021). The histotroph, uterine lumen fluid or also called uterine milk, is the only source of vitamins, minerals, enzymes, hormones, cytokines, growth factors, protein carriers, amino acids and other components that the fetus needs before implantation, these come from the synthesis and secretion of the cells of the uterus specifically epithelial and stromal cells (Jamwal et al., 2024). It also serves as a source of biomarkers of uterine function to understand the environment in which the embryo develops in its early stages. When the embryo implants, communication occurs between the mother and the fetus through the fetal membranes (Galina, 2021).

From this moment on, the embryo is nourished directly by the mother, which is called hemotroph, and becomes the main source of nutrients. Likewise, the chorioallantoic placenta is formed (Galina, 2021), which transports nutrients, gases, and waste products between the mother and the fetus. This exchange of nutrients occurs between the fetal epithelial barrier and the fetal blood vessels. In particular, the uterus of ruminant animals contains caruncles, which are glandular areas of stroma covered with luminal epithelial cells, which intertwine with the villi of the fetal cotyledons to create a structure called a placentoma through which the easily diffused nutrients that are necessary for the development of the fetus are transferred (Dunlap et al., 2015).



Source: Alvarez-Oxiley, 2022

Figure 3. Stages of embryonic development up to implantation.

2.3. OXIDATIVE STRESS AND ITS IMPACT ON REPRODUCTION

2.3.1 Oxidative stress

In all living organisms, there are molecules called ROS (Reactive Oxygen Species), which contain oxygen and are by-products of cellular respiration and normal metabolism. Although their production is necessary to provide feedback between metabolic activity and the regulation of cellular functions, excessive production causes damage to the body. There are also other molecules, such as reactive nitrogen species, superoxide, hydroxyl and peroxyl radicals, nitrogen oxide, and peroxynitrite, which are generated by the body, although in small quantities (Rakha et al., 2022; Sa'ayinzat et al., 2021). Oxidative stress is generated by an imbalance in redox reactions, i.e., between the oxidizing molecules that are produced from aerobic metabolism and protective antioxidants (Adeoye et al., 2017; Sa'ayinzat et al., 2021). Redox reactions involve the transfer of electrons between molecules while aerobic respiration and metabolic activities are carried out, as cells respond to certain stimuli, both endogenous and exogenous, and when they receive them, byproducts are generated, such as reactive species, which are oxygen and nitrogen free radicals, which ends up in oxidative stress for the body (Sa'ayinzat et al., 2021). Free radicals, also called reactive oxygen metabolites, are highly reactive and damage biological macromolecules such as proteins, lipids, carbohydrates, and nucleic acids (Nikolova et al., 2022). Reactive oxygen species can come from endogenous sources, such as mitochondria, peroxisomes, and NADOH oxidases or NOX, as well as from exogenous sources, such as ultraviolet light, excessive heat or cold, pollutants, and nutrient deficiencies (Meulmeester et al., 2022).

2.3.2 Consequences on eggs, sperm, embryos, and placenta

Free radicals affect the entire body; however, in the reproductive part, it affects oocytes, spermatozoa, and the development and implantation of the embryo (Adeoye et al., 2017). In the development and function of gametes, physiological levels of ROS are necessary; however, when the production of these molecules is excessive, oxidative stress is generated for the organism that triggers cell destruction, DNA damage, and low or no growth (Rakha et al., 2022).

In the early stages of pregnancy, the immune system undergoes a change in the mother's body as it decreases immune cells to give the embryo a chance to implant (Jiménez et al., 2024). In turn, the metabolism of the placenta and embryo increases the number of macrophages and neutrophils in the blood and phagocytes, which triggers the production of reactive oxygen species, but surprisingly, the antioxidant activity begins to be activated (Jiménez et al., 2024).

A low amount of metabolic reserves generates adverse stimuli where an imbalance in redox levels occurs, that is, the body suffers oxidative stress. These changes in the redox system directly influence fitness outcomes (Hao et al., 2021; Pintus & Ros-Santaella., 2021). However, evidence has shown that not only the low metabolic reserves offered in feed are the cause of oxidative stress, but also that animals face the ravages of climate change, which leads them to suffer from heat stress, causing low expression in development, reproduction and health (Pal et al., 2024), since this factor alters the epidermis growth factor signaling pathway in the endometrium shortly after estrus, in addition pregnancy rates are altered, in turn stromal cells increase their production of prostaglandins F2α and alters the production of chemokines (Amelkina et al., 2025). Therefore, a promising strategy is supplementation with antioxidants, vitamins, and minerals such as vitamin A, E, selenium, copper, and zinc (Pal et al., 2024).

2.4. ROLE OF SELENIUM IN REPRODUCTION

2.4.1 Selenium

Selenium is a trace element considered essential for its antioxidant, anti-inflammatory, antimutagenic, antiviral, antibacterial, antiparasitic, and anticancer activities. Its distribution in nature is limited, as it is found in only a small percentage of the Earth's crust. However, in all layers of the earth, specifically in agricultural soils, the amount of selenium varies between 0.33 and 2 mg kg-1 worldwide, which represents a deficiency in the forages consumed by animals. However, in mineral soils with higher rock content, such as limestone and sandstone, selenium concentration also increases. In addition, this element is also found in atmospheric air from volcanic gases, in groundwater in the form of selenates and selenites, as well as in seawater, although this concentration occurs due to runoff from excessive fertilization of agricultural soils, water resulting from oil refining and coal combustion residues (Hosnedlova et al., 2017; Grzybowska et al., 2023; Díaz-Zarco et al., 2022; Mojadadi et al., 2021; Ma et al., 2012). From the beginning of its discovery (1817), selenium was considered a toxic element; it was not until years later (1957) that its benefits were discovered and that it was named a trace element, essential for the body. Curiously, selenium can be coupled to elements such as hydrogen, bromine, chlorine, fluorine, and phosphorus, generating products analogous to those containing sulfur, since selenium shares similar properties with sulfur (Mojadadi et al., 2021).

In animals, selenium is stored in muscles and kidneys, but to perform its functions, it must be incorporated as selenocysteine and selenomethionine, which are then integrated into proteins and function throughout the cell; these are called selenoproteins. Although not all selenoproteins have been fully studied and understood, their importance is undeniable. Thyroid hormone deiodinases have been recognized as regulators of thyroid hormone metabolism. Selenoprotein P has been found to function as a selenium transporter to all peripheral tissues, while thioredoxin reductases and glutathione peroxidases (GPx) function as antioxidants (Grzybowska et al., 2023; Hosnedlova et al., 2017; Mojadadi et al., 2021; Mesalam et al., 2023; Kuria et al., 2021).

Glutathione (GSH) is a thiol tripeptide composed of three amino acids: cysteine, glycine, and glutamine. This is the main sulfhydryl component present in mammalian cells that maintains the redox state within the cell and, by interacting with its associated enzymes glutathione peroxidase and reductase, facilitates its protective action (Adeoye et al., 2017). In animals, glutathione peroxidase catalyzes the reduction of hydrogen peroxide and lipid peroxide in the presence of glutathione in its reduced form, which is converted to glutathione in its oxidized form. The damage that can be caused by the lack of this enzyme (GPx) is the deterioration of cell membranes, an increase in the intracellular concentration of calcium, as well as an interruption of Ca2+ channels and therefore damage to cellular mitochondria, ending in apoptosis or cell death (Grzybowska et al., 2023; Hosnedlova et al., 2017; Adeoye et al., 2017; Yao et al., 2016). A function that only the enzyme glutathione peroxidase performs is the prevention of lipid peroxidation, as it reduces lipid peroxides to alcohols. In turn, they regulate redox reactions that generate reactive oxygen species (ROS), which are destructive to cells (Grzybowska et al., 2023).

Selenium has been found to be necessary for thyroid hormone synthesis and participates in the conversion of thyroxine (T4), which is a precursor to triiodothyronine (T3), into its active form, which is involved in the regulation of amino acid metabolism, reproduction, as well as muscle circulation and function (Ziaei, 2015; Vaswani & Kumar, 2023; Daghigh et al., 2019). Selenium deficiency causes muscular dystrophy, also known as the well-known white muscle disease. In chickens, white muscle disease due to selenium deficiency was replicated, and pathological and molecular changes, including oxidative stress, cell death, disordered selenoproteins, mitochondrial damage, and Ca2+ leakage, were observed (Yao et al., 2016). In turn, low concentrations of these elements have been found to cause disorders such as dietary hepatosis and morera heart disease in pigs, encephalopathies, exudative diathesis in birds, and myopathies in ruminants and horses (Nikolova et al., 2022). Another function of selenium is the protection of cells from damage caused by heavy metals, such as mercury and lead, through its antagonistic mechanism (Ziaei, 2015; Mesalam et al., 2023).

Selenium also participates in the synthesis and methylation of DNA, promotes angiogenesis by ensuring the proper delivery of nutrients to the oocyte, and supports the immune response. On the other hand, it has been found that selenium deficiency alters systems and organs not only in females but also in males (Ramírez, 2018). Selenium in males improves testosterone synthesis and therefore spermatogenesis (González-Rodríguez et al., 2018). Selenium is also thought to influence enzymes involved in carbon metabolism, as it does in regulating microRNAs (Dahlen et al., 2022).

2.4.2 Influence on reproduction: progesterone, placenta, and embryonic development

The addition of selenium, in either organic or inorganic form, improves reproductive traits (González-Rodríguez et al., 2018), but supplementing the mother with selenium enhances her offspring's health and protects them against oxidative stress. Selenium increases the percentage of fertile oocytes and follicle development (López, 2021) and promotes oocyte maturation. This mineral also influences granulosa cells and stimulates the production of 17-β-estradiol, which favors increased ovulation rates (Daghigh et al., 2019). In the ovaries of healthy cows, high amounts of selenium have been found specifically located in the preovulatory follicles, indicating that this element is in direct contact with the preovulatory oocyte and therefore plays a preparatory role for ovulation and subsequent fertilization.

It has also been observed that adequate amounts of this mineral facilitate the transport of sperm within the female reproductive tract (Dahlen et al., 2022) as it improves the contractions of the uterus (Susilowati et al., 2024), so when semen is deposited in the female's reproductive tract, selenium generates an anti-inflammatory response and shows a protective role in ensuring fertilization (Dahlen et al., 2022; Jiménez et al*.,* 2024). Once egg fertilization is achieved, blastocyst formation and quality are improved with selenium supplementation (González-Rodríguez et al., 2018). In vitro studies carried out on various animals have shown that the addition of selenium has been shown to have good results in the survival, implantation, and development of the embryo (Dahlen et al., 2022; Jiménez et al*.,* 2024), and decreases embryonic death in the first month of gestation (López, 2021; Mehdi & Dufrasne, 2016). In cattle, selenium supplementation has been observed to increase elongation of the bovine conceptus (Dahlen et al., 2022). On the contrary, deficiencies of this mineral can have serious consequences, resulting in fertility failure, silent estrus, ovarian cysts, embryonic death during implantation, abortions, retained placenta, poor immunity, and great weakness in neonates (Ziaei, 2015; Susilowati et al., 2024).

Selenium is also involved in the function of the placenta, the growth, and the development of the fetus's nervous system (González-Rodríguez et al., 2018). The selenium requirements that the fetus demands are covered transplacentally, this depends on the amount that the mother has of the mineral in her body, but in ruminants, selenium transport occurs even when the mother has little availability of the mineral, so it is inferred that the mother could sacrifice her requirements to maintain a correct transport of selenium to the fetus (Carbajal et al., 2012). Its supplementation during early pregnancy promotes an increase in lung, hepatic, visceral mass, there is a greater formation of blood vessels in the small intestine which is probably because the mother must increase her metabolic capacity to be able to support the demands of the fetus, there is also an increase in the vascular development of the mammary gland and therefore in the production and composition of colostrum. Surprisingly, the same thing also occurs in the fetus as it grows, increasing its body, lung, cardiac, visceral, and skeletal muscle cell mass and density (Dahlen et al., 2022).

The application of selenium to sheep during gestation demonstrated an increase in cell proliferation in the placenta (Dahlen et al., 2022). Interestingly, glutathione peroxidase enzymes and selenoprotein-P are found to a greater extent in the placenta. Very specifically, in mice it has been observed that these proteins allow the transport of selenium from the embryo to the placenta so that this transport ensures the protection of the body during gestation (Jiménez et al., 2024), on the contrary, mothers with selenium deficiency during pregnancy decrease the activity of glutathione peroxidase and thioredoxin reductase in the placenta, An important temporary organ in the maintenance of pregnancy, which causes a restriction in the growth of the fetus or premature births. This deficiency is evidenced by increased H_2O_2 content and reduced GSH levels in this organ (Cheng et al., 2022).

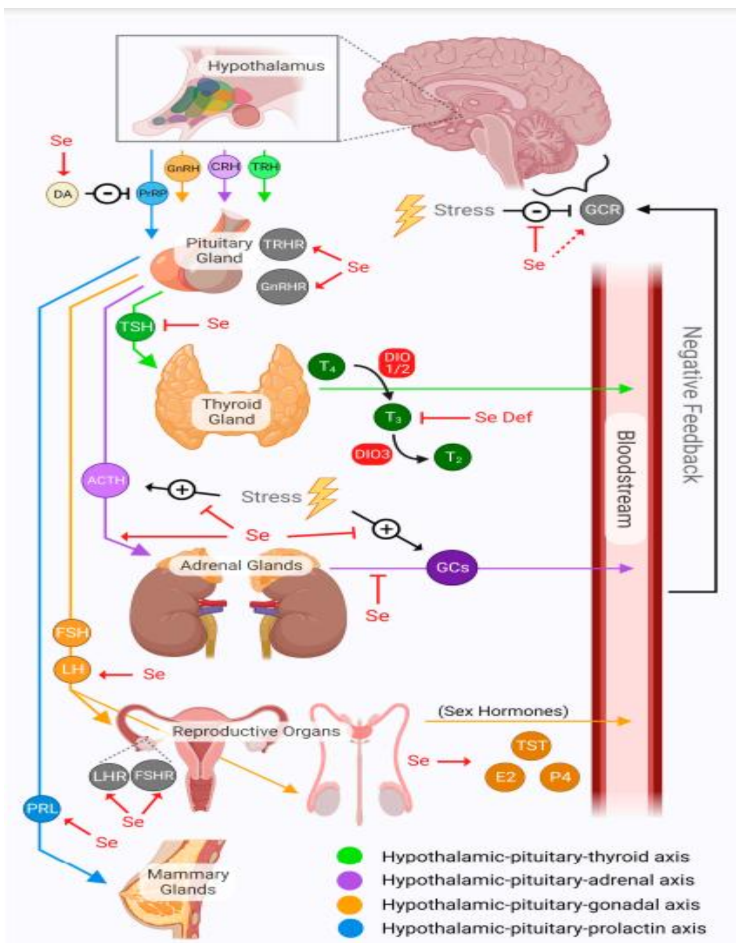
The placenta produces hormones such as progesterone, which is essential for the success of pregnancy, and it has been observed that when a mixture of organic and inorganic selenium is added, genes related to maternal recognition of pregnancy are stimulated, showing an increase in progesterone concentrations in the luteal phase, which favors both embryo elongation and the production of tau interferon (Carr et al., 2023; Jiménez et al., 2024; Dahlen et al., 2022). Dairy cows supplemented with selenium showed a higher progesterone concentration not only in the early phase of pregnancy but also at an advanced stage, and after calving, this increase was maintained compared to non-supplemented cows. Crites et al. (2022) conclude that P4 concentration is higher when a mixture of organic and inorganic selenium is used than when only inorganic selenium is used, but this difference may be due to the number of animals used for treatment.

When the amount of progesterone remains high after ovulation, the quality of the embryo improves, which assumes that implantation will be successful (Crites et al., 2022), on the contrary, it has been seen that a negative regulation of selenoproteins suppresses progesterone biosynthesis in the placenta by reducing the expression of the Hsd3b1 gene and decreasing the expression of nutrient transporters, this according to transcriptome analysis carried out in mice (Cheng et al., 2022), however, another explanation could respond to this behavior, as research on mRNA transcripts in the corpus luteum showed an increase in Ldlr and Hsl transcripts that participate in the regulation of cholesterol availability in the body, so it is inferred that the increase in progesterone in the corpus luteum is not due to a direct effect on it but is a consequence of the availability of cholesterol when supplemented with selenium. The LRP8 receptor binds SELENOP to deliver selenium to the cell and participates in cholesterol homeostasis (Toh et al., 2022).

On the other hand, when cows are supplemented with selenium, fewer ovarian cysts are observed, the incidence of placental retention decreases, and the anestrus season is shortened (Mehdi & Dufrasne, 2016). Other research on water buffaloes confirms the results described above: selenium and vitamin E supplementation during pregnancy alleviate oxidative stress at this stage (Nikolova et al., 2022). The maximum recommended levels in goats can vary from 0.7 mg per animal per day to 0.3 ppm in the total ration (Ziaei, 2015), as excess of this element in the body can cause toxicity, malformations in embryos, and inhibition of their growth rate (Ma et al., 2012; Ramírez, 2018). This is confirmed by the results of Ramírez (2018), who concluded that the application of inorganic selenium decreased progesterone concentrations, resulting in reduced fertility and embryo implantation. However, this response may be due to the amount administered, suggesting that it caused toxicity.

2.4.3 Selenium in immune function

In recent years, it has been seen that selenium is highly involved in both animal and human nutrition, exerting immune responses in several species through the activation of phagocytosis by neutrophils, increasing the production of antibodies, and developing the proliferation of lymphocytes (Pérez, 2016), this immunity is characterized by a transfer between the mother and the fetus (Pérez 2016). It has been observed that there is an acute increase in defense proteins that act against bacteria and fungi (Jiménez et al., 2024). López (2021) concludes that selenium decreases the number of neutrophils and basophils after blastocyst formation and four days after selenium is applied.



Source:Toh *et al*., 2022.

Figure 4. Pathways of action of selenium.

Low intakes of microbial and viral infections decrease resistance to these infections, which compromises the proper function of neutrophils, antibody production, T and B lymphocyte proliferation, and NK cell cytotoxicity. Selenium also appears to modulate inflammatory and immune processes through oxidoreduction functions (Surai & De Marco, 2021). Selenium has other functions, such as thermogenesis, which help prevent decreases in immune defenses due to both viral and bacterial infections and improve nutrient metabolism (Pérez, 2016). Selenium is necessary for the proper functioning of immune system cells, including neutrophils, macrophages, NK cells, and T lymphocytes (Surai & De Marco, 2021). According to Campos-Granados (2015), supplementing ruminants with vitamin E (3000 IU) per day during the transition period did not allow a decrease in neutrophil reproduction or interleukin 1 (IL-1) production. Likewise, the chemotactic response of neutrophils was not affected after delivery. It was reported that supplemented calves had higher antibody titers, which allowed them to cope with various diseases (Campos-Granados, 2015).

2.5. ROLE OF VITAMIN E IN REPRODUCTION

2.5.1. Vitamin E

Vitamin E (Vit E) was discovered in 1922, and its importance in reproduction was studied in pregnant rats (Traber, 2021). Vitamin E is a powerful fat-soluble antioxidant that creates protection against lipid peroxidation caused by free radicals, but not only protects but also eliminates free radicals very effectively, it is essential for the functions it performs such as its enzymatic activity and neurological processes, it has been observed that when there are deficiencies physiological and immunological functions are compromised (Grzybowska et al., 2023; Wang et al., 2017; Mesalam et al., 2023; Tedeschi et al., 2021). Vit-E prevents peroxidation in susceptible subcellular membranes and oxidative damage to sensitive lipid membranes by decreasing hydrogen peroxide production, thereby promoting membrane integrity and reducing oxidative stress. Vit-E donates phenolic hydrogen to peroxyl lipid radicals to convert them into α-tocopheroxyl radicals, thus becoming less harmful (Ziaei, 2015; Mesalam et al., 2023; Moghimi-Kandelousi et al., 2020). In recent years, the α-tocopherol transfer protein (α-TTP) was discovered; this protein facilitates the secretion of α-tocopherol from the liver into the circulation (Traber, 2021).

Khan et al. (2016) showed that when dairy cows are supplemented with Vit-E (1000 IU/cow/day), cortisol and heat shock protein (HSP 70) levels decrease considerably, without differences between breeds. Regarding the concentration of progesterone, it increases in treated cows regardless of the breed, on the contrary, and very interestingly, the number of follicles had a decrease in the four breeds used, however, the authors suggest that both the initial body condition, the period of supplementation and the dose administered are factors that could affect the results (Sahiwal, Achai, Mestizos and Holstein-Friesian) (Khan et al., 2016).

Both cortisol and progesterone are synthesized from cholesterol; therefore, higher cortisol levels decrease P4 levels. Therefore, Vit-E can reduce stress levels, as shown by Khan et al. (2016). Dønnem et al. (2015) suggest that Vit-E supplementation leads to a positive relationship between serum α-tocopherol and cholesterol, but when delivery occurs, cholesterol levels decrease. In addition, the benefits of Vit-E are also linked with the selenium-containing enzyme glutathione peroxidase (GPx) (Dønnem et al., 2015).

Vit-E is essential in reproduction as it counteracts the effects of oxidative stress on eggs and sperm (González-Rodríguez et al., 2018). This trace element protects steroidogenic enzymes from oxidative damage, in turn, it has been seen that when Vit-E is added, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and adrenocorticotropic hormone (ACTH) increase (Khan et al., 2016), on the other hand, it has been found that, in infertile organisms, the concentrations of Vit-E and other antioxidants are found in low concentrations (Wang et al., 2017).

It seems that Vit-E, along with other vitamins and components, is a compound found in oxygen scavengers present in follicular and oviduct fluids, protecting embryos from oxidative damage and improving embryo development before implantation. The use of vitamins, such as E, to improve embryo development is becoming increasingly important, even in vitro, as their effectiveness in blastocyst cryopreservation across different animal species has been demonstrated (Deluao et al., 2022; Sarbandi et al., 2021). To assess the viability of preimplantation embryos, mitochondrial function is essential, as it provides the energy required for this process; however, surprisingly, α-tocopherol also contributes to mitochondrial metabolism, directly and indirectly influencing embryo development (Sarbandi et al., 2021).

Vit-E addition in the transition period in cows considerably decreases perinatal mortality, due to better placental function (Moghimi-Kandelousi et al., 2020; Dønnem et al., 2015). Adding Vit-E to the diet increases angiogenesis, or the formation of new blood vessels in the placenta, suggesting that increased blood vessel formation improves nutrient supply and fetal development (Dønnem et al., 2015). Although studies in nutrigenomics are still in their incipient stages, Vit-E has been observed to overexpress the FSCN3 gene but to negatively regulate the genes CYP26B1, NDRG1, TUBB, and certain types of collagen. All these results led to spermatogenesis and an increase in the number of germ cells in the epididymis (Kizilaslan et al., 2022).

Vit-E enhances immunity by increasing immunoglobulin G production (Dønnem et al., 2015) and inducing neutrophil-mediated killing of intracellular microorganisms at the time of delivery. Leukocyte membranes contain more free fatty acids, which makes them more susceptible to vitamin E (Khan et al., 2016). The total Vit-E intake in ewes at the end of pregnancy is 504 IU/day/animal, regardless of the number of fetuses (Dønnem et al., 2015).

2.5.2 Selenium - Vit-E interaction

The results of a regression meta-analysis confirm the synergy that exists between Vit-E and selenium during reproduction by increasing fertility rates (Moghimi-Kandelousi et al., 2020), stimulating the estrogenic process, increasing the secretion and release of gonadotropins, and triggering increased folliculogenesis (Daghigh et al., 2019); however, these studies are still few, suggesting an area of opportunity in the field of research (Moghimi-Kandelousi et al., 2020). Other effects of the interaction between selenium and Vit-E include inhibition of fatty acid oxidation and a reduction in plasma cholesterol (Daghigh et al., 2019). The synergy between these elements improves estrus frequency, pregnancy rate, calving percentage, and birth weight, and reduces both mortality rates and the incidence of placental retention-related problems (Zubair et al., 2015; Alalaf & Alnuaimy, 2024).

Both Vit-E and selenium are powerful antioxidants; however, they should be used according to the recommended doses as excess of these can cause hair loss, muscle tremors, dizziness, myocardial attacks, kidney failure, and breathing difficulties (Alalaf & Alnuaimy, 2024). Vit-E deficiency can lead to ataxia due to neuronal death in the peripheral nervous system (Traber, 2021).

2.6. LITERATURE CITED

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CHAPTER III

3.1. ARTICLE

SELENIUM + VITAMIN E SUPPLEMENTATION IN ANESTRUS GOATS: A STRATEGY TO ENHANCE REPRODUCTIVE OUTCOMES UNDER A SEMI-ARID PRODUCTION SYSTEM

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Selenium + Vitamin E Supplementation in Anestrus Goats: A Strategy to Enhance Reproductive Outcomes Under a Semi-Arid Production System

Raquel Santos-Silva [1], Cesar A. Meza-Herrera [1,*], Brenda Castro-Roque [1], Guadalupe Calderón-Leyva [2,*], Cayetano Navarrete-Molina [3], Oscar Angel-García [2], Jessica M. Flores-Salas [2], Angeles De Santiago-Miramontes [2], Fernando Arellano-Rodriguez [2] and Cesar A. Rosales-Nieto [4]

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Simple Summary: Seasonal reproduction in goats leads to production seasonality, presenting physiological and economic challenges for producers who aim to extend the reproductive season to ensure a more consistent supply of products and year-round economic returns. Within this complex biological, physiological, and economic context, precision nutritional supplementation to enhance out-of-season ovarian activity in females appears to be a promising strategy. This study evaluated the possible effect of selenium (5 mg) plus vitamin E (350 IU) supplementation (i.e., SeVE) on ovarian activity, embryo implantation, and pregnancy rates in estrus-induced anestrous goats. SeVE supplementation increased ($p < 0.05$) estrus induction, ovulatory rate, embryo implantation, and pregnancy rates compared to the control group, without affecting ($p > 0.05$) corpus luteum diameter or left and right ovary diameters.

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Abstract: Goats are primarily raised in marginal, arid, and semi-arid production systems and exhibit a seasonal reproductive pattern. Extending their reproductive season would enhance food security and improve the economic viability of goat production. Therefore, the objective of this study was to assess the effects of SeVE supplementation during the anestrus season on ovarian activity in anestrous goats subjected to an estrus synchronization protocol. The response variables included estrus induction (EI), ovulatory rate (OR), corpus luteum diameter (CLD), left and right ovary diameters (LOD and ROD), embryo implantation (EMBRYO), and pregnancy rates (PREG). Adult, anestrous, crossbred dairy goats (n = 32) from northern semi-arid Mexico (i.e., Comarca Lagunera, 26° N) were randomly assigned to two homogeneous groups based on age, live weight (LW), and body condition score (BCS): (1) the supplemented group (SeVE; n = 16), received 5 mg selenium plus 350 IU vitamin E, and (2) the control group (CONT; n = 16), received physiological saline. Estrus was induced using a combination of progesterone (P4, 20 mg), equine chorionic gonadotropin (eCG, 400 IU), and exposure to the male effect. While no differences ($p > 0.05$) were observed between the groups for LW and BCS, the variables EI, OR, EMBRYO, and PREG rates were higher ($p < 0.05$) in the SeVE group than the CONT group. No differences ($p > 0.05$) were observed between the groups for CLD, LOD, or ROD. Thus, SeVE-targeted supplementation improved reproductive outcomes without affecting ovarian structure size (i.e., a non-dimensional ovarian SeVE action), suggesting that this precision supplementation strategy certainly enhances reproductive efficiency. This was possibly related to selenium-induced increases in P4 synthesis, and the antioxidant effects on follicular development correlate with improved endometrial development, conceptus survival, embryo growth, and pregnancy maintenance. Such improvements could contribute to the sustainability of marginal production systems in the semi-arid regions of northern Mexico, supporting food security and increasing economic returns for goat producers and their families.

Keywords: goats; anestrous; male effect; targeted supplementation; reproductive efficiency; food security; zero hunger

1. Introduction

Goat production is a vital agricultural activity that has contributed significantly to human development, particularly in marginal, arid, and semi-arid ecosystems and rural communities facing poverty. In these regions, goats provide employment opportunities and a critical source of animal protein for families, often representing their primary or sole source of income [1–4]. Goats exhibit remarkable adaptability across diverse agroecological zones, owing to their ability to withstand droughts and extreme climates, combined with desirable traits such as high prolificacy, rapid growth rates, and efficient utilization of low-quality plant resources [2,3]. In northern arid Mexico, goat production remains a cornerstone of rural economies, accounting for a substantial proportion of farmers' income [5]. As of 2023, Mexico reported a goat population of approximately 9 million head, with the Comarca Lagunera (i.e., CL; 26° N) standing out as the leading goat-producing agroecological region, housing 404,414 goats [6]. The CL, a semi-arid region, is recognized nationally for its high goat milk production. However, pronounced seasonal fluctuations in vegetation and crop residue availability have resulted in a seasonal reproductive pattern, subsequently driving a seasonal production cycle for both milk and meat [7].

This reproductive seasonality imposes biological constraints that limit the sustainability of marginal goat production systems [8]. Goat reproduction is primarily regulated by photoperiodic variations, with a period of reproductive quiescence—marked by the absence of estrus and ovulatory activity—extending from January to early June. Reproductive activity typically resumes in late June and continues through December [9]. This pattern presents a significant physiological challenge for goat farmers seeking to extend the reproductive season to ensure more continuous production [10]. In this complex biological framework, the strategic use of socio-sexual cues (i.e., the male effect) combined with precision nutritional supplementation can enhance the metabolic status of females and facilitate the reactivation of the hypothalamic–pituitary–gonadal axis, thereby inducing out-of-season ovarian activity [10]. Consequently, there is an urgent need to identify targeted supplementation strategies to boost reproductive efficiency [11].

The use of vitamins and minerals has emerged as a promising supplementation strategy to enhance animal productivity and reproductive performance, given their role as enzymatic cofactors in multiple metabolic pathways [12]. Selenium (Se) and vitamin E (VE), in particular, act synergistically to exert antioxidant effects through independent biochemical mechanisms [13]. These antioxidants protect cellular membranes from oxidative damage induced by reactive oxygen species and lipid hydroperoxides [14], thereby preserving the integrity of DNA, RNA, and proteins, and preventing cell death by reducing oxidative stress [15]. Improvements in growth, reproductive performance, immune competence, hormone synthesis, and tissue integrity have been associated with adequate Se and

VE status [14]. Specifically, sufficient blood Se levels have been linked to enhanced progesterone secretion [16], improved corpus luteum development [17], and reduced lipoperoxide concentrations [18]. Moreover, Se and selenoproteins (i.e., SeProt) are involved in lipid metabolism (i.e., cholesterol-to-progesterone) due to their ability to modulate redox homeostasis. In the last processes, some SeProt encoding genes (i.e., GPX1, GPX4, SELENOP, and SELENOF) and the redox-related gene (i.e., SOD2) were involved [19]. Additionally, SeVE supplementation has been shown to improve ovarian function, mitigate morphological changes, and minimize oxidative damage to ovaries and follicles [20–23]. SeVE has also been associated with a shortened postpartum estrus interval [17] and plays a crucial role in the activation of key enzyme systems such as glutathione peroxidase (GSH-Px) and deiodinases, both essential for optimal embryonic development [24]. These effects collectively contribute to higher pregnancy rates and increased kidding percentages [25]. Given that oxidative stress during seasonal anestrus may further suppress ovarian activity, the antioxidant properties of SeVE could mitigate this constraint by enhancing both follicular resilience and pregnancy rates. Besides, and from a social and environmental standpoint, enhancing goat reproductive efficiency aligns with several Sustainable Development Goals (SDGs), notably contributing to food security (SDG 2) and promoting responsible production practices (SDG 12) [2,26,27]. Building on these previous findings, we hypothesized that precision supplementation with SeVE would improve out-of-season reproductive performance in adult anestrus crossbred dairy goats in northern semi-arid Mexico, leading to increased ovarian activity, embryo implantation, and pregnancy rates. The present study was designed to test this hypothesis.

2. Materials and Methods

2.1. General

All experimental procedures, animal handling, and management practices in this study were conducted strictly within international [28] and national [29] guidelines for the ethical treatment, care, and welfare of research animals. While all the reported procedures were aligned with conventional veterinary practices, the study received institutional approval under the reference number UAAAN-UL-38111-425501002-2876.

2.2. Location and Environmental Conditions

The study was conducted in the ejido Lázaro Cárdenas, San Pedro, Coahuila de Zaragoza, Mexico (25°45′50″ N, 103°11′03″ W; altitude 1111 m above sea level). The region is characterized by a very dry, semi-warm climate, with rainfall concentrated between May and October; the annual precipitation ranges from 200 to 300 mm [30,31]. Average annual temperatures are approximately 24 °C, with summer highs reaching 41 °C and winter lows dropping to −1 °C. The study was carried out in two phases: (1) the male-to-female interaction phase during April and May, corresponding to the natural seasonal anestrus period, and (2) the implantation, embryogenesis, and early gestation phase during June and July.

2.3. Animal Management and Rangeland Conditions

Multiparous, non-lactating, non-pregnant, anestrous, crossbred goats (i.e., Criollo × Alpine–Saanen–Nubian; n = 32) of known fertility were selected from a 160-goat herd managed under a interesting semi-intensive production system subdivided according to their stage of lactation. In this peculiar production system, those goats that had just given birth and those in the 1/3 and 2/3 lactation (i.e., ≈9 months on lactation) were kept under an intensive management scheme (i.e., zero grazing) with free access to alfalfa hay, mineral salts, and water, while receiving 200 g day^{-1} goat^{-1} of a commercial concentrate

(14% CP) (see Table 1). On the other hand, the goats in the 3/3 period of lactation, or all through their dry period, were kept under a semi-intensive management approach consisting of a diurnal grazing scheme with evening confinement with reduced nutritional support. In both corrals/groups, the goats were hand-milked daily in the morning; in the second group, milking was carried out before grazing. Grazing was allowed from 1000 to 1800 h in the grazing areas surrounding the production unit, with the guidance of a herdsman. The rangeland has flora characterized as Chihuahuan desert rangeland; while creosote bush (*Larrea tridentata* (DC. Cov)) dominates the grazing area, different key species consist of lechuguilla (*Agave lechuguilla* Torr), mesquite (*Prosopis glandulosa* v. glandulosa), and blue gramma (*Bouteloua gracilis* (Wild). Ex Kunth Lag. Ex Griffiths). Goats walk approximately 5–8 km daily from the pen to diverse sites of the available rangeland, thus grazing restraints can be considered negligible. Once in the pen, after the grazing period, all 32 goats received 200 g of alfalfa hay and 100 g of a commercial concentrate (14% CP), day^{-1} $goat^{-1}$, with free access to mineral salts and water (see Table 1), during the whole experimental period. Since all the goats received such nutritional support on an individual basis and grazed the same rangeland, the goats were considered the experimental unit. The selected goats were homogeneous regarding live weight (LW; 39.4 ± 1.3 kg), body condition score (BCS 1.7 ± 0.0 units, scale: 1 = very thin to 4 = very fat), and age (2.4 ± 0.3 years old) to ensure balanced groups before randomization. To confirm anovulatory status during the increasing photoperiod (April, Northern Hemisphere; Figure 1), ovarian activity was evaluated by an expert using two transrectal ultrasound examinations (Aloka SSD 500, Tokyo, Japan, 7.5 MHz transducer) conducted seven days apart [32,33].

Table 1. Chemical composition of alfalfa hay, commercial concentrate, and mineral salts that comprised the basal diet in the adult multibreed goats supplemented with selenium + vitamin E (SeVE; *n* = 16) or control (CONT; *n* = 16) under natural photoperiod conditions during the anestrus season in northern Mexico (25° N).

Item	Alfalfa Hay [a]	Commercial Concentrate [b]	Mineral Salts [b]
Nutrient Composition	**(%)**	**(%)**	**(%)**
Dry matter	92.8	- -	- -
Crude protein	15.5	14.0	- -
Neutral detergent fiber	56.9	- -	- -
Acid detergent fiber	41.1	- -	- -
Crude Fat	- -	6.8	- -
Crude Fiber	- -	2.0	- -
Humidity	- -	10.8	- -
Ash	- -	5.5	- -
Nitrogen-Free Extract	- -	62.9	- -
Phosphorus	0.26	- -	9.0
Calcium	1.71	- -	8.5
Magnesium	0.20	- -	1.2
Sodium	0.12	- -	- -
Chlorine	0.35	- -	- -
Potassium	2.16	- -	- -
Iron	- -	- -	740 ppm
Manganese	- -	- -	2030 ppm
Zinc	- -	- -	2600 ppm
Copper	- -	- -	520 ppm
Iodine	- -	- -	96 ppm
Cobalt	- -	- -	12 ppm
Selenium	- -	- -	10 ppm

[a] Composition values were determined according to the procedures outlined by AOAC, 2023 [34]. [b] Nutritional values correspond to the guaranteed analysis declared by the manufacturer.

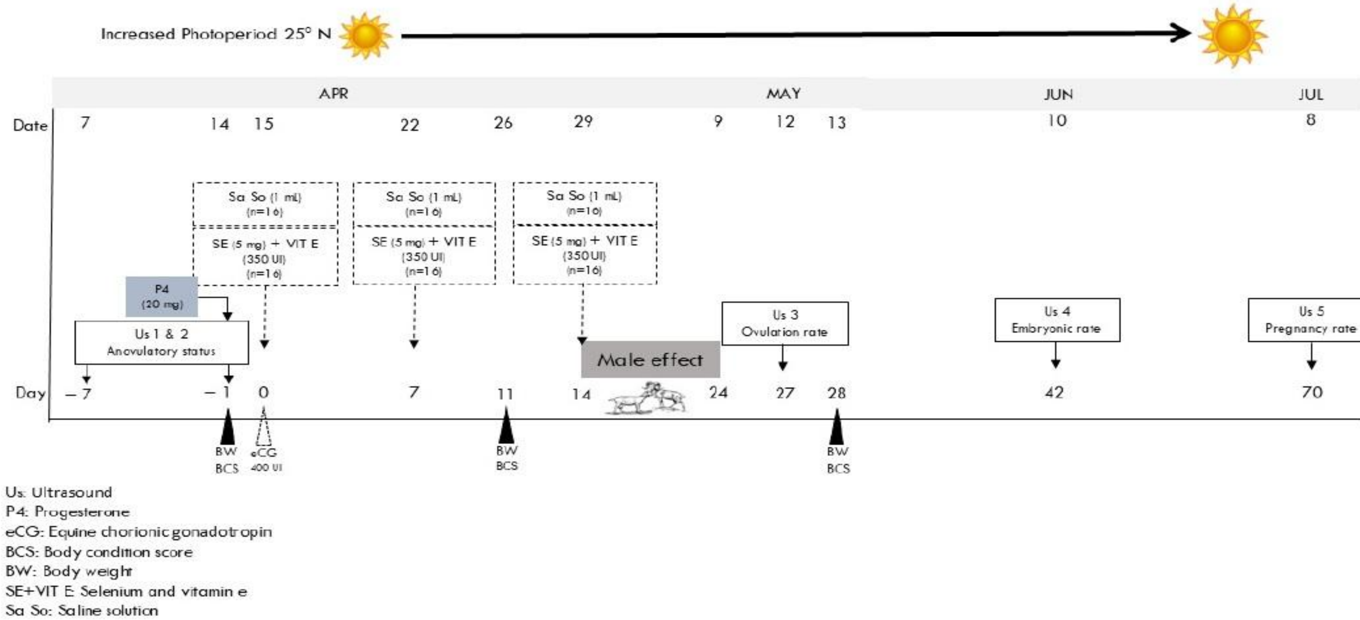

Figure 1. A schematic overview of the experimental timeline is presented. Two ultrasounds (US 1 and 2) were performed before estrus induction (following P4 and eCG administration), and subsequent evaluations (US 3, 4, and 5) assessed ovulation rate, ovarian activity, embryo development, and pregnancy status during the seasonal anestrus period at 25° N latitude.

2.4. Experimental Procedures, Experimental Groups, and Treatment Design

Goats showing no evidence of follicular development (n = 32) were selected for estrus induction. Estrus was induced via intramuscular injection of 20 mg progesterone (P4; Progestelas "E", Lab Aranda, Mexico City, Mexico), followed 24 h later by 400 IU equine chorionic gonadotropin (eCG; Foligon, MSD, Animal Health, Mexico City, Mexico) administered intramuscularly. This was designated as Day 0 of the experimental protocol. Then, goats were randomly assigned to two experimental groups: Supplemented group (SeVE; n = 16) received three intramuscular applications of 5 mg selenium plus 350 IU vitamin E (i.e., SELEBEN-B, Maymo Lab., S.A., Madrid, Spain) administered at 7-day intervals. Control group (CONT; n = 16), received three intramuscular applications of 1 mL physiological saline solution at 7-day intervals. Following treatments, sexually active males were introduced to both experimental groups for 10 days to induce estrus via the "male effect" (Figure 1) [35,36].

2.5. Measurements and Response Variables

2.5.1. Corporal Measurements

Live weight (LW) and body condition score (BCS) were recorded at three points: at the beginning of the experiment, 12 days after initiation, and one day after the third ultrasound evaluation. Body condition was assessed by palpating the lumbar region and scoring on a scale from 1 (very thin) to 4 (very fat).

2.5.2. Determination of Ovarian Size, Structures, and Activity

Thirteen days after male introduction, a transrectal ultrasound (Aloka SSD 500, Tokyo, Japan, 7.5 MHz transducer) was performed to assess ovarian structures in each goat. Estrus induction was determined by calculating the percentage of goats presenting a corpus luteum (i.e., CL) relative to the total number of goats per group. Additionally, the number and diameter of corpora lutea and the left and right ovary diameters were recorded.

2.5.3. Embryonic Rate and Pregnancy Percentage

At 28 days post-male introduction, a fourth ultrasound (Aloka SSD 500, Tokyo, Japan, 7.5 MHz transducer) examination was conducted to detect embryonic vesicles and quantify the number of embryos per female. Embryo implantation rate was calculated as the number of embryos divided by the number of ovulated females per group. Pregnancy confirmation was performed at 56 days post-male introduction via a fifth ultrasound evaluation.

2.6. Statistical Analyses

At enrollment, goats were blocked based on LW and BCS. The response variables of attributes such as LW and BCS were analyzed by a completely random design split-plot analysis of variance for repeated samples in the same animal across time. Response variables included estrus induction response (EI), corpus luteum number (OR) and diameter (CLD), left and right ovary diameter (LOD and ROD), embryonic implantation rate (EMBRYO), and pregnancy rate (PREG). Since both treatment application and response variable quantification were individually collected in both experimental groups, the goat was considered the experimental unit. Thus, the fixed effects treatments as well as sampling date were assessed using a MIXED model for repeated measures across time, with time as the repeated measure and treatment as the repeated subject, regarded as the random error term. Estrus induction (i.e., induced or not) as well as pregnancy rate (i.e., pregnant or not) were analyzed using the generalized linear mixed model procedures with a binomial distribution and logit link function (PROC-GLIMMIX); treatment was the fixed effect. Least-squares means and standard errors for each class of treatments, sampling time, and their interaction were computed; mean comparisons were solved by means of Fisher's least significant difference. All statistical analyses were done using the procedures of the SAS statistical package version 9.2; a significant difference between means was set at $p < 0.05$.

3. Results

Table 2 summarizes both experimental groups' corporal, ovarian, and reproductive responses. No differences ($p > 0.05$) were found between groups for initial live weight (LW; 41.78 ± 1.08 kg) and body condition score (BCS; 1.69 ± 0.04 units) at the beginning of the breeding period. Similarly, no differences ($p > 0.05$) were observed for corpus luteum diameter (CLD; 9.1 ± 1.5 mm), left ovary diameter (LOD; 16.6 ± 1.4 mm), or right ovary diameter (ROD; 16.3 ± 1.3 mm). However, key reproductive variables—estrus induction (%), ovulation rate (units), number of embryos (units), and pregnancy rate (%)—were higher ($p < 0.05$) in the SeVE-supplemented group compared to the control group. Specifically, the SeVE group exhibited increases of 43.75%, 35.00%, 28.50%, and 41.33% over the control group values, respectively. These results indicate that SeVE supplementation improved ovarian activity (i.e., ovulation rate) and enhanced reproductive outcomes (i.e., estrus induction, embryo implantation, and pregnancy rate), without affecting ovarian structure size, suggesting a non-dimensional morphological SeVE's action.

Table 2. Least squares means ± standard error for live weight (LW, kg), body condition score (BCS, units), estrus induction (EI, %), ovulation rate (OR, units), corpus luteum diameter (CLD, mm), left ovary diameter (LOD, mm), right ovary diameter (ROD, mm), embryo number (EMBRYO, units), and pregnancy rate (PREG, %) in adult multibreed goats supplemented with selenium + vitamin E (SeVE; n = 16) or control (CONT; n = 16) under natural photoperiod conditions during the anestrus season in northern Mexico (25° N).

Variables	SeVE	CONT
LW, kg	42.04 ± 1.67 [a]	41.53 ± 1.43 [a]
BCS, units	1.72 ± 0.06 [a]	1.66 ± 0.06 [a]

Table 2. *Cont.*

Variables	SeVE	CONT
	Ovarian Size and Activity	
EI, %	80.0 ± 11 [a]	35.0 ± 12 [b]
OR, units	1.20 ± 0.22 [a]	0.42 ± 0.22 [b]
CLD, mm	9.1 ± 1.1 [a]	9.2 ± 1.9 [a]
LOD, mm	16.5 ± 1.2 [a]	16.8 ± 1.7 [a]
ROD, mm	15.6 ± 1.1 [a]	17.0 ± 1.5 [a]
	Embryo Number and Pregnancy Rate	
EMBRYO, units	1.23 ± 0.20 [a]	0.35 ± 0.19 [b]
PREG, %	75.0 ± 11.4 [a]	31.2 ± 11.2 [b]

[a,b] Values on the same line with different superscripts differ ($p < 0.05$).

4. Discussion

Globally, goat production represents a critical source of income and food security for smallholders, particularly in arid and semi-arid regions where other livestock species face significant production constraints. This study tested the hypothesis that SeVE supplementation enhances reproductive performance by increasing ovarian activity, embryo implantation rates, and pregnancy rates in crossbred goats during the anestrus season. The results support this hypothesis: SeVE-supplemented goats exhibited higher values for key reproductive variables without differences in live weight, body condition, or ovarian diameter relative to controls. Therefore, our working hypothesis is not rejected. Interestingly, despite these reproductive enhancements, ovarian structures and corpus luteum diameters remained unaffected. These findings highlight SeVE supplementation as a viable strategy for advancing sustainable livestock production and contributing to global development goals related to responsible production and food security.

At the onset of the experiment, anestrus status was confirmed in all the goats. Following treatment, the SeVE group achieved an 80% estrus induction rate, consistent with previous studies using 10–20 mg P4 and 100 IU eCG administered 24 h after P4 in goats during the anestrus season [37]. The administration of eCG after P4 appears to shorten the time to follicular growth, likely mimicking the luteinizing hormone (LH) surge [38–40]. Moreover, a 20 mg P4 dose is sufficient to eliminate short, infertile cycles, enabling effective estrus synchronization and onset of ovarian activity [37]. Females receiving estrus induction protocols supplemented with SeVE achieved 100% induction efficacy [41], likely reflecting an additive or synergistic effect of SeVE on follicular responsiveness and ovulatory outcomes [25,39,42,43].

Regarding ovulation rate—defined as the number of corpora lutea—the SeVE group showed higher values ($p < 0.05$), suggesting that SeVE supplementation during anestrus enhances follicular development and ovulation even with short-term administration [44]. Selenium supplementation has been reported to stimulate ovarian activity by promoting follicular growth and increasing ovulation rate (i.e., corpus luteum formation) [45]. This effect is partly mediated through enhanced cholesterol availability for progesterone (P4) biosynthesis, with the LRP8 receptor facilitating selenium–protein interactions critical for cholesterol homeostasis [46].

Although no direct link between selenium and GnRH has been identified, selenium may influence LH activity by upregulating GnRH receptor expression in the anterior pituitary, potentially increasing LH synthesis [46]. LH promotes ovarian estrogen release, ovulation, and luteal development [47]. Additionally, selenium may enhance ovarian vascularization, contributing to improved follicular and luteal function [48,49]. In cattle, selenium accumulates in granulosa cells of preovulatory follicles and is associated with corpus luteum development [50]. Furthermore, selenium's antioxidant role protects corpus luteum function by mitigating oxidative damage [51]. Consistent with prior studies, ovarian size did not differ between groups, suggesting that short-term SeVE supplementation impacts ovarian function rather than structural dimensions [50–52].

Embryo implantation and pregnancy rates were also significantly higher in the SeVE-supplemented goats. Selenium is known to promote favorable reproductive tract changes that facilitate sperm transit, fertilization, and implantation [53,54]. Selenium enhances uterine architecture and function by reducing epithelial detachment and increasing myometrial mass [55]. Moreover, selenium-induced increases in P4 synthesis correlate with improved endometrial development, conceptus survival, embryo growth, and pregnancy maintenance [25,56,57]. Synchronization between the embryo and the maternal endometrium prior to implantation is critical. Selenium supplementation has been linked to differential gene expression in maternal recognition of pregnancy, particularly those responsive to INFtau and P4 [53]. Using a blend of organic and inorganic selenium (i.e., 35 ppm) has demonstrated improvements in endometrial and conceptus gene expression [58]. Moreover, preventive SeVE supplementation has improved pregnancy rates post-embryo transfer in heifers (64.7% vs. 41.2%); the latter denotes an interesting cross-species relevance in ruminants [59].

Despite these positive effects, some studies report neutral or negative outcomes of selenium supplementation. For example, no differences in reproductive efficiency were observed in some trials [53,54], possibly due to adequate baseline nutrition, which masks supplementation effects. Similarly, no differences in LW or BCS were observed between groups in this study, aligning with prior findings in goats [60,61] and ewes treated with Se and vitamin E during estrus synchronization [25]. In contrast, excessive or long-term selenium supplementation may impair blastocyst quality and implantation success due to tissue selenium accumulation and embryotoxicity [62]. Therefore, assessing baseline feed mineral content before supplementation is crucial, in addition to tailoring interventions to the physiological stage, timing, dose, and administration route to optimize outcomes [63].

Undeniably, the results obtained represent practical and accessible options for goat producers not only from the biological, productive, and economic perspectives, but also in marginal contexts. In fact, the SeVE group showed a 43.8% improvement in the pregnancy rate, which corresponds to an increase in offspring of 0.7 per goat. If the cost per treatment (i.e., SeVE) is equivalent to USD 1.83 per goat and the selling price for a kid is around USD 51.16 (i.e., MXN 1000), revenues increase up to MXN 35.81 per goat. This means that SeVE treatment shows a cost–benefit ratio of 19.57; if producers invest USD 1, they receive USD 19.57. The above considerations are based on the sale of meat alone; such scenarios can rise significantly if additional revenues are derived from an increasing milk volume in dairy goat production systems.

5. Conclusions

The obtained results support our working hypothesis that short-term precision supplementation with selenium and vitamin E in adult crossbred dairy goats improves key reproductive variables, such as estrus induction, ovulation rate, embryo implantation, and pregnancy rate. Such enhancements occurred with no significant differences between the experimental groups' live weight or body condition scores. Similarly, no variations occurred in the diameters of the left and right ovaries or the corpus luteum diameters. Thus, a short-term supplementation may not achieve the requested Se level to potentially affect some "long-term response variables" (i.e., live weight, body condition score, and ovarian size–morphology), but it will undoubtedly affect other "short-term response variables" (i.e., estrus induction (i.e., d27), embryo implantation rate (i.e., d42), and pregnancy rate (d70). Improving reproductive outcomes could help not only to stabilize goat producers' and their families' year-round income but could also contribute to higher animal protein supply and greater resilience in marginal goat production systems. Undoubtedly, longitudinal studies to evaluate the economic sustainability and scalability of our research findings, are certainly pending assignments.

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Informed Consent Statement: Informed consent was obtained from the animal owner involved in the study.

Data Availability Statement: None of the data were deposited in an official repository, yet information can be made available upon request.

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Abbreviations

The following abbreviations are used in this manuscript:

SeVe	Selenium and vitamin E	P4	Progesterone
Se	Selenium	eCG	Equine chorionic gonadotropin hormone
VE	Vitamin E	CONT	Control group
EI	Estrus induction	CL	Comarca Lagunera
OR	Ovulatory rate	DNA	Deoxyribonucleic acid
CLD	Corpus luteum diameter	RNA	Ribonucleic acid
LOD	Left ovary diameter	GSH-Px	Glutathione peroxidase
ROD	Right ovary diameter	CP	Crude protein
EMBRYO	Embryo implantation	Us	Ultrasound
PREG	Pregnancy percentage	SaSo	Saline Solution
LW	Live weight	LH	Luteinizing Hormone
BCS	Body condition score	GnRH	Gonadotropin-releasing hormone

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CHAPTER IV

4.1 GENERAL CONCLUSION

Goat production is a viable activity that provides meat, milk, and economic sustenance for many families living in climatically vulnerable regions such as arid lands; however, this vulnerability entails reproductive seasonality. This seasonality is influenced by the photoperiod and lack of food, causing prolonged periods of anestrus, restricting reproductive efficiency, and generating a marked seasonality of its products. In this context, the success of key processes such as embryo implantation is central as it determines the establishment and maintenance of pregnancy. That is why the identification of precision nutritional strategies that improve reproductive functionality during anestrus is essential to increase the sustainability and profitability of production systems.

The results obtained show that selenium + vitamin E supplementation is an effective tool to improve embryo implantation and reproductive performance in goats during seasonal anestrus. Targeted supplementation of these micronutrients showed positive effects on embryo implantation, which proves to have a functional action. This effect must be aligned with the improvement of the body's antioxidant status, an increase in progesterone synthesis, and the optimization of the uterine environment, which causes a synchronous action between the embryo during the implantation stage.

The implementation of targeted nutritional strategies or precision supplementation, such as the use of selenium + vitamin E, not only represents an effective biological alternative to improve embryo implantation and reproductive efficiency in goats, but it also becomes a tool with a high social and economic impact for goat farmers, especially those located in vulnerable regions. The last should allow for greater productivity and economic stability while strengthening food security and improving the quality of life of goat keepers and their families.