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**ADDIS ABABA UNIVERSITY**  
**COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE**  
**DEPARTMENT OF CLINICAL STUDIES**

**GRADUATE PROGRAM IN VETERINARY GYNAECOLOGY AND OBSTETRICS**

**EVALUATION ON THE MODULATION EFFECT OF HIGH PROTEIN AND ENERGY  
DIET ON ESTRUS CHARACTERISTICS, PROLIFICACY, AND PREGNANCY RATE  
IN MENZ BREED SHEEP**

**MSc THESIS RESEARCH**

**BY:**

**ANIMAW ANDARGIE WORKU**

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**BISHOFTU, ETHIOPIA**



**ADDIS ABABA UNIVERSITY  
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE  
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**Graduate Program in Veterinary Gynaecology and Obstetrics**

**Evaluation on the Modulation Effect of High Protein and Energy Diet on Estrus  
Characteristics, Prolificacy, and Pregnancy Rate in Menz Breed Sheep**

**MSc Thesis Research**

**By:**

**Animaw Andargie Worku**

**Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science  
(MSc) in Veterinary Gynaecology and Obstetrics.**

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**June, 2024**

**Bishoftu, Ethiopia**

## APPROVAL SHEET

This is to confirm and approved that this thesis paper entitled as “**Evaluation on the Modulation Effect of High Protein and Energy Diet on Estrus Characteristics, Prolificacy and Pregnancy Rate in Menz Breed Sheep**” submitted in the partial fulfilment of the requirements for the award of the Degree of Master of Science (MSc) in “Veterinary Gynaecology and Obstetrics” to the Graduate Program of Addis Ababa University College of Veterinary Medicine and Agriculture by Animaw Andargie Worku is an authentic work carried out by him under our supervision and guidance. We can approve without hesitation that the matter embodied in this project work has not been submitted earlier for an award of any degree or diploma to the best of our knowledge and belief.

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As members of the Board of Examiners of the Master of Sciences (MSc) thesis open defence examination; we have read and evaluated this thesis prepared by Animaw Andargie Worku entitled “**Evaluation on the Modulation Effect of High Protein and Energy Diet on Estrus Characteristics, Prolificacy and Pregnancy Rate in Menz Breed Sheep**” We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (MSc) in Veterinary Gynaecology and Obstetrics.

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## **DEDICATION**

This dissertation is dedicated with profound love and reverence to my dear parents, Andargie Worku and Menen Belachew, whom I lost when I was just a baby. My Dad and Mom though, our time together on this earth was tragically cut short, the imprint you left on my life is indelible.

You may have been taken from me before I could truly know you, but your spirit, values, and unwavering love have been the guiding light that has led me to this moment. I only wish I could have had more years to learn from you, to be nurtured by you, and to make you proud.

This achievement is as much a testament to your memory as it is to my own hard work. I hope that wherever you are now, you can feel the depth of my gratitude and the fullness of my love for you both.

May you rest peacefully, secure in the knowledge that your child has honored your memory in the best way I know how.

## **STATEMENT OF THE AUTHOR**

I declare that this thesis is my own original work. All sources used in this thesis have been properly acknowledged. This thesis has been submitted to Addis Ababa University as partial fulfillment of the requirements for my MSc degree, and a copy has been deposited in the university library to be made available to borrowers. I solemnly affirm that this thesis has not been submitted to any other institution for any academic award, diploma or certificate. Brief quotes from this thesis are permitted with proper citation, without needing specific authorization. The Dean of Postgraduate Studies or the Head of the Department of Clinical Studies may grant permission for extensive quotations or partial/full replication of this work if they believe it advances scholarly purposes. However, consent from the author is required for any other use cases.

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## LIST OF ABBREVIATIONS

AR	Androgen Receptor
CGE	Clean, Green and Ethical
CL	Corpus Luteium
DHT	Dihydrotestosterone
E <sub>2</sub>	Esterogen
EDTA	Ethylene Diamine Tetra Acetic Acid
FSH	Follicular Stimulating Hormone
FSHR	Follicular Stimulating Receptor
GCs	Granulosa Cells
GluH <sub>3</sub>	Glucose Hexokinase <sub>3</sub> Cells
GnRH	Gonadotropin Releasing Hormone
IGF-I	Insulin like Growth Factor-I
LH	Luteinizing Hormone
LHR	Luteinzing Hormone Receptor
P <sub>4</sub>	Progesterone
PGF <sub>2</sub> $\alpha$	Prostaglandin F <sub>2</sub> -alpha
RPM	Rotation Per Minute

## ABSTRACT

This study aimed to evaluate the effect of short-term nutritional supplementation on the body weight, onset and duration of estrus, pregnancy rate, prolificacy, and plasma glucose level in Menz breed ewes. Twenty-four healthy and normally cycling ewes were distributed to two treatment groups T1: (Lupin; n=8), T2; (Barley; n=8) and one control group C: (Control; n=8) using randomized block design. Estrus was synchronized with double prostaglandin injections given 9 days apart to achieve the target late luteal phase of estrous cycle. Grass hay was provided as the basal diet for ewes in all treatment and control groups. In addition to grass hay, ewes were allowed to graze for 8 hours during the day time, and they had free water access. Ewes in the treatment group T1 were supplemented with 500g of Lupin grain per head per day, divided into 250g in the morning and 250g in the evening. Similarly, ewes in treatment group T2 were supplemented with 500g of Barley grain, also divided into 250g portions for morning and evening. The supplementation lasted for 9 consecutive days during the late luteal phase of the estrous cycle. The average weight of ewes did not vary between or within the groups ( $P > 0.05$ ). The mean time to onset of estrus (mean $\pm$ SD) varied significantly ( $P < 0.05$ ) between the treatment groups T1 (29.25 $\pm$ 2.88), T2 (31.5 $\pm$ 2.95), and the control group C (42 $\pm$ 1.96). The supplemented groups T1 and T2 accelerate the ewes to come into estrus earlier than non-supplemented control group (C). The duration of estrus (mean $\pm$ SD) also varied significantly ( $P < 0.05$ ) between the treatment groups T1, T2, and the control group (C). The non-supplemented control group, exhibited the shortest estrus duration (29.13 $\pm$ 1.44), followed by treatment group T2 (35.63 $\pm$ 2.71) and T1 (41.5 $\pm$ 2.19). The supplemented groups of ewes demonstrated an extended duration of estrus than the non-supplemented control group. The plasma glucose concentration revealed a significant difference ( $P < 0.05$ ) across all groups and the duration of supplement administration. The pregnancy rate varied significantly ( $P < 0.05$ ) between the groups, with the rate of 100% in Lupin supplemented group, 87.5% in Barley supplemented group, and 50% in non-supplemented Control group. The prolificacy rate was also varied significantly ( $P < 0.05$ ) with the rate of 50% in Lupin supplemented groups of ewes, followed by the Barley supplemented group (13%) and 0% in non-supplemented Control group. It could be concluded that Lupin supplementation improves response to estrus, plasma metabolic profile, pregnancy and prolificacy rate in ewes. We recommend a detailed study on the effect of Lupin on reproductive hormones and more other blood metabolites.

**Keywords:** *Bishoftu, Ethiopia, Menz ewes, Nutritional supplement, Pregnancy rate, Prolificacy.*

## 1. INTRODUCTION

Sheep, among other livestock animals play a key role in the world's economy because of their high production traits (Ma *et al.*, 2017; Al-Thuwaini, 2021). From all sheep production systems value, the litter size is considered as a valuable quality (Yavarifard *et al.*, 2015; Abd-Allah *et al.*, 2019). Additionally, a key factor in flock efficiency is reproductive performance (Mazinani and Rude, 2020).

Sheep are an important part of Ethiopia's livestock production, distributed across various agro-ecologies in the country (Abebe *et al.*, 2023). The country has 42.9 million sheep; of this, approximately 70.05% are indigenous breeds (CSA, 2020). Despite the large population of sheep, their performance is hampered by inadequate and poor-quality feed, disease and parasites, poor infrastructure, and the lack of organized breeding programs and policies (Tesfaye *et al.*, 2023).

Reproductive ability in sheep is commonly measured by fertility, prolificacy, and fecundity (Abdoli *et al.*, 2016) which also has a direct consequence on profitability of the farms. Fertility is described as the number of lambing per year, while prolificacy is litter size, and fecundity refers to the number of lambs produced per year per ewe. Ovulation rate and litter size are important reproduction traits in sheep with high economic merit (Notter, 2008).

Nowadays, the concept of clean, green, and ethical (CGE) management has gained traction as a blueprint for the growth of the animal industries especially for sheep industries because it emphasizes animal welfare, environmental effect, and chemical input reduction (Martin *et al.*, 2004; Simões *et al.*, 2021). The two main CGE management strategies used by the sheep industry are "focus feeding" for the control of reproductive performance and the "ram effect" to control when ovulation and lambing occurs (Martin *et al.*, 2004; Delgadillo and Martin, 2015). For vast and subsistence production systems, where the expenditures of prolonged periods of high-level feeding can be prohibitive, focus feeding is an appealing choice (Delgadillo and Martin, 2015).

Three elements determine a sheep flock's reproductive efficiency: fertility, prolificacy, and lamb survival (Viñoles, 2003). Prolificacy, which is determined by ovulation rate, is a key factor in reproductive efficiency that can be improved by nutrition. Nutrition is one of the most significant external factors influencing fertility in small ruminants (Murawski *et al.*, 2021). Effect of

nutrition on the ovulation rate in sheep has been recognized as: the static, dynamic, and intermediate effect (Viñoles, 2003; Scaramuzzi *et al.*, 2006). The static effect refers to the higher ovulation rate in heavy weight ewes compared with lighter once. The dynamic effect is referred to as a rise in the ovulation rate due to an increase in live weight and body condition score shortly before the estrous period and mating. The intermediate effect is referred to as the short-term supplementation with a high-energy, high-protein diet resulting in a higher ovulation rate without any significant changes in live weight or body condition score (Viñoles, 2003).

There is a positive correlation between feeding management and the reproductive parameters of small ruminants, such as sheep (Scaramuzzi *et al.*, 2006). Flushing, which refers to a deliberate and brief rise in the nutrient plane around the breeding season, is one technique for enhancing sheep reproduction success. The quantity of blood metabolites, such as glucose, increases during nutritional flushing and are essential for the physiological processes of folliculogenesis and then ovulation (Archer *et al.*, 2002; Zabuli *et al.*, 2010).

Follicular populations in sheep, are highly responsive to dietary input, allowing for easy modulation of folliculogenesis and ovulation rate (Scaramuzzi *et al.*, 2006). Nutrition is an affordable management tool to control ovulation rate and litter size in sheep (Martin *et al.*, 2004). It does this by modulating circulating metabolic hormones like glucose, insulin, insulin-like growth factor I (IGF-I), and leptin, which all affect the reproductive and physiological functions (Armstrong *et al.*, 2003).

### **1.1. Statement of the Problem**

The stark difference between the amount of potential output and the number of lambs born suggests that sheep reproduction is a very inefficient process. If every potential egg in a ewe's ovaries reached maturity, she could yield about 40,000 lambs. In contrast, a herd of sheep only gives birth to five to ten lambs throughout her lifetime. Although all sheep breeds contain large volumes of waste, the differences in lambing outcomes indicated that management can affect sheep's reproductive performance (Joshi, 2022).

There are critical periods in the life of ewes that can dictate fertility and prolificacy and it is possible to monitor nutrition closely during these critical periods in order to optimize the ewes' reproductive success (Fernandez, 2013). Currently, synchronizing the estrous cycle to focus on

the crucial late luteal phase is the basis of short-term nutritional treatments to boost ovulation rate in ewes (Gulliver, 2014)

One of the biggest challenges facing the sheep industry is still low ovulation rates, which lead to a low incidence of multiple births (Somchit-Assavacheep, 2011). Litter size is influenced by nutrition level in that inadequate nutrition during the service period lowers ovulation rates and increases embryonic death, which in turn reduces litter size (Abate, 2016). The percentage of ewes having twins in tropical sheep breeds, generally ranges between 0 and 50% (Abate, 2016) while under traditional management conditions the percentage tends to fall even below 10%. According to Edea (2008) a twinning rate of 39.9 % or litter size of 1.40 and 36 % or litter size of 1.36 were obtained for Bonga and Horro sheep breeds, respectively, whereas low twinning rate of 8% and 13% or litter size of 1.08 and 1.13 was reported for Menz sheep breed by Edea (2008) and Mukasa-Mugerwa *et al.* (2002) respectively.

Strategic feeding consists of short-term supplementation with diets high in protein and energy to change the metabolic and nutritional status of the ewes and improve their ovarian potential (Hernández-Marín *et al.*, 2022). This study hypothesizes that the naturally low prolificacy rate known in Menz breed ewes can be modulated using a short term nutritional supplementation and this approach is also believed to positively influence the metabolic profile, estrus response, and hence the pregnancy rate of Menz breed ewes.

## **1.2. Objectives of the Study**

- To evaluate the effect of short-term nutritional supplementation on pregnancy success rate and prolificacy in Menz sheep breeds.
- To evaluate onset and duration of estrus and body weight changes in response to focused feeding plane.
- To investigate the effects of feeding a high-protein, high-energy diet on the plasma concentrations of glucose.

## 2. LITERATURE REVIEW

### 2.1. Reproductive Cyclicity of Ewe

The ewe is thought to be a seasonal polyoestrous species with an annual reproductive cycle that includes a distinct breeding and non-breeding season (Abecia *et al.*, 2012). A series of neuroendocrine events that start during the breeding season eventually lead to ovulation, but these events stop during the non-breeding season, often known as the seasonal anoestrus period (Dobson *et al.*, 2012).

Changes in photoperiod play a major role in controlling the transition between reproductively active and inactive phases. Sheep are referred to as short-day breeders (Abecia *et al.*, 2012) and when the numbers of hours of darkness increases, the pineal gland secretes the hormone, melatonin. Melatonin then causes the release of gonadotropin-releasing hormone (GnRH), a hormone that regulates reproduction and restores cyclicity (Gulliver, 2014). But, in tropical regions with minimal photoperiod fluctuation, ewes sustain their cyclic behaviour virtually throughout the year (Arroyo *et al.*, 2007; Arroyo, 2011). The ewe ovulates roughly every 16 to 17 days during the breeding season, giving her numerous chances to mate and become pregnant (Gulliver, 2014).

#### 2.1.1. *Hormonal dynamics during estrous cycle*

It is widely known that hormones generated by the hypothalamus (GnRH), pituitary gland (FSH, LH), follicles in the ovary ( $E_2$ , inhibin), corpus luteum ( $P_4$ , oxytocin), and the uterus ( $PGF_{2\alpha}$ ) interact intricately to regulate the estrous cycle. Responses to both internal and external stimuli, such as food supply and photoperiod are swiftly resolved by homeostatic feedback systems (figure 1) (Gulliver, 2014).

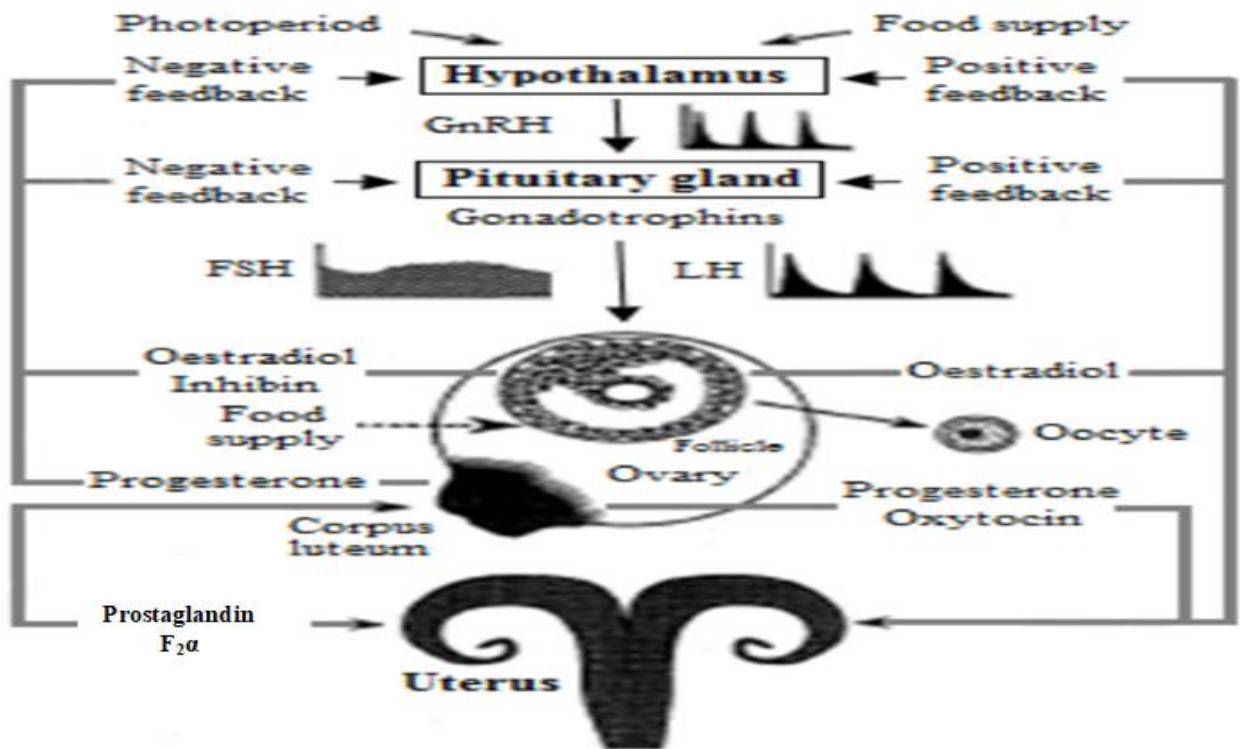


Figure 1: Diagram illustrated the interaction between the reproductive structures, hormones and feedback system regulating estrous cycle of ewes. Where GnRH=Gonadotropin-releasing hormone; FSH=Follicular stimulating hormone and LH= Luteinizing hormone. Adapted from Gulliver (2014).

### 2.1.2. Phases and stages of the estrous cycle

The luteal and follicular phases are the two separate phases that make up the estrous cycle (figure 2) (Gulliver, 2014).

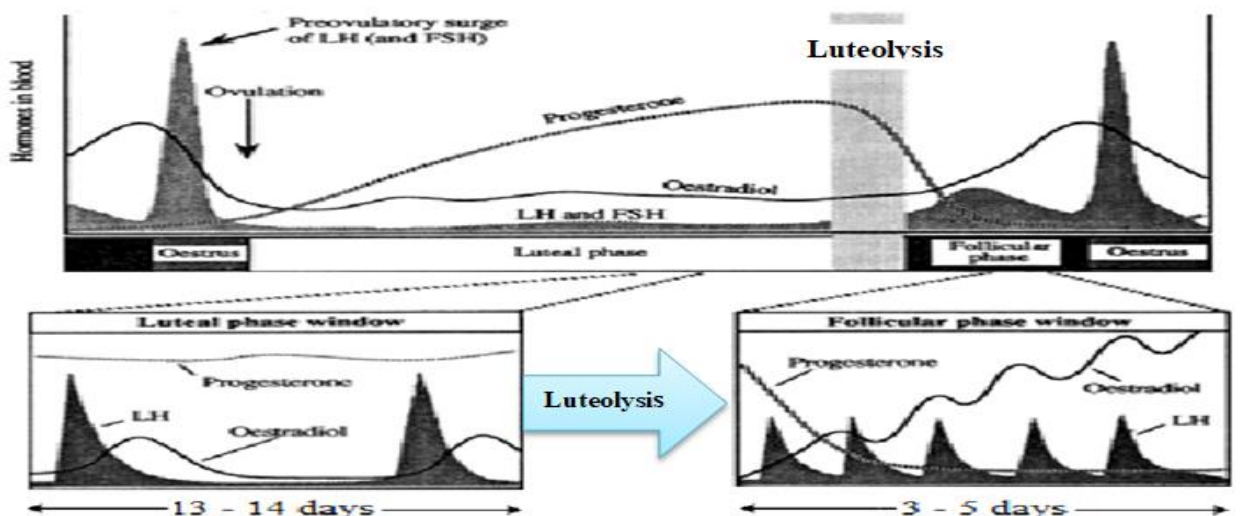


Figure 2: Schematic representation showing, the two phases of estrous cycle and the major endocrine events involved in the estrous cycle of ewes. Adapted from Gulliver (2004).

### ***The follicular phase***

With duration of about three to five days, the follicular phase is the shorter of the two phases of the estrous cycle. Proestrus and estrus are the two stages that make up this phase. The first stage, known as proestrus, lasts for two to five days and is characterized by the formation of ovarian follicles that secrete E<sub>2</sub> (Bartlewski *et al.*, 2011). Oestradiol initiates estrus behaviour in the ewe, prepares the female reproductive system for mating, and controls the onset of pre-ovulatory surge of LH which stimulates ovulation (figure 2). Estrus is the other stage which lasts 1 to 2 days (typically between 24 and 36 hours). The egg's spontaneous ovulation, which occurs 30-36 hours after the start of estrus, occurs during sexually receptive period and estrus behaviour displayed by ewe during this time (Gulliver, 2014).

When P<sub>4</sub> levels are low at the beginning of the follicular phase due to luteolysis of the corpus luteum, which secretes P<sub>4</sub>, the pulse frequency of GnRH being rise and the anterior pituitary releasing FSH and LH (Bartlewski, 2011). Gonadotropins then promote growth and recruitment follicles from ovaries which release E<sub>2</sub> by promoting their development. E<sub>2</sub> first gives the hypothalamus positive feedback to keep secreting the gonadotropins necessary for follicle development. However, once blood levels of E<sub>2</sub> cross a particular threshold, E<sub>2</sub> give the anterior pituitary negative feedback to reduce the amount of FSH released (figure 3). At this stage, the dominant follicle also releases inhibin. Inhibin together with E<sub>2</sub> suppresses the release of FSH and eliminates the FSH dependent follicles (Bartlewski *et al.*, 2011).

LH pulse frequency starts to rise in response to increasing E<sub>2</sub> levels as FSH secretion declines. This pre-ovulatory gonadotrophic surge works to trigger ovulation by causing alterations in the ovarian follicle. This signals follicular phase end up by causing the rupture of follicles and release an ovum (Gulliver, 2014). This phase is characterized by the rapid development of follicles. However, the growth of follicles is not limited only within this narrow window of time. Rather, it is a protracted cyclical process which finally starts even in the growing foetus and sustained for the duration of reproductive life of ewes (Gulliver, 2014).

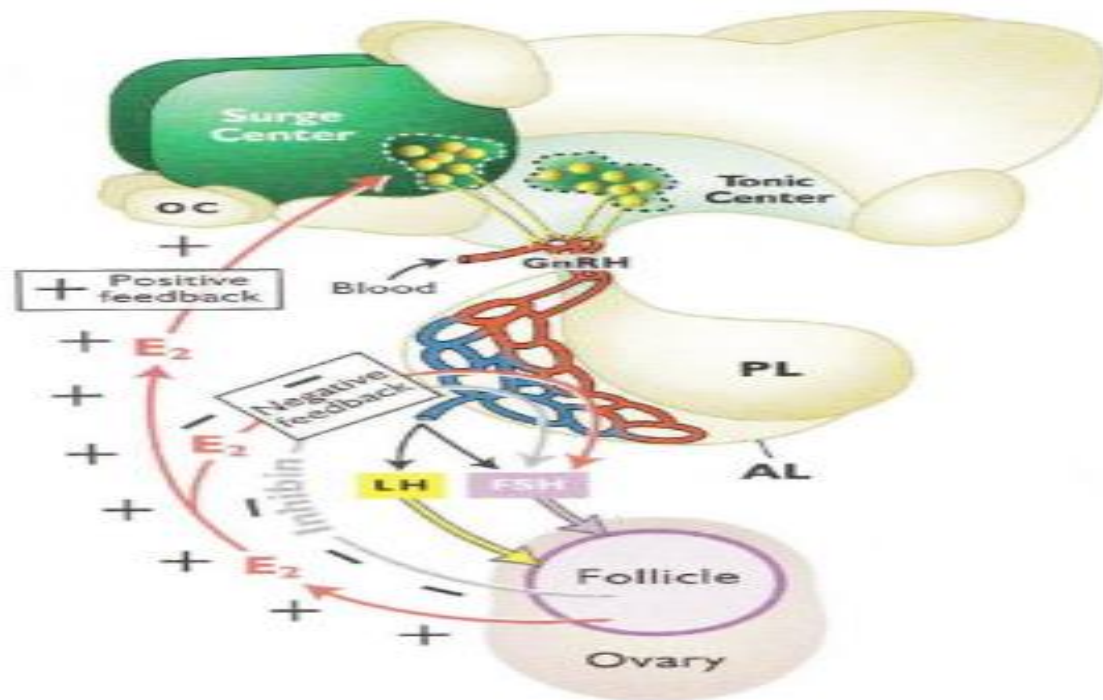


Figure 3: The Relationship between the hypothalamus, the pituitary and the ovaries occurring during the follicular phase of estrous cycle of ewe. Where AL=Anterior pituitary; E<sub>2</sub> Estrogen; OC Optic chiasm and PL= Posterior lobe of pituitary. Adapted from Senger (2003).

### *The luteal phase*

The luteal phase is the longer of the two phases which lasts 13-14 days and it begins following to ovulation (Gulliver, 2014). Again this phase comprised of 2 stages, metestrus and diestrus. Metestrus characterized by the development of corpus luteum from ovarian follicls after ovulation and this stage lasts 2-5 days. Diestrus is the second and longer stage which lasts 10-14 days and at stage corpus luteum secrete P<sub>4</sub> and oxytocin hormones (Gulliver, 2014). Progesterone helps the reproductive system get ready for pregnancy, while oxytocin is crucial for luteolysis process (Hafez *et al.*, 2000).

Luteal phase comes to an end with luteolysis. The ewe cannot return to the follicular phase in the absence of pregnancy until luteolysis has taken place. Luteolysis happens over 1-3 day time period at the end of luteal phase. For the occurrence of effective luteolysis, the corpus luteum and uterine endometrium communicated well. Hormones secreted from the corpus luteum (oxytocine and P<sub>4</sub>) and endometrium of uterus (PGF<sub>2</sub>α) play the role of this communication (Bartlewski *et al.*, 2011).

To release PGF<sub>2</sub>α, the uterus has to be exposed to increased levels of p<sub>4</sub> for about 10-12 days (Gulliver, 2014). P<sub>4</sub> levels at this time can be elevated enough to prevent the uterus from developing oxytocin receptors. However, P<sub>4</sub> loses its capacity to prevent the development of these receptors after 10 to 12 days, allowing oxytocin receptors in the uterine endometrium to proliferate. The uterus rises the amplitude and pulse frequency of PGF<sub>2</sub>α production after a critical quantity of oxytocin is identified, starting luteolysis (Gulliver, 2014).

### 2.1.3. Folliculogenesis and ovulation

Folliculogenesis is a dynamic process that happens continually throughout the estrous cycle and involves follicular development, maturation, and degeneration. Just a Very few primordial follicles advance the pre-antral and antral stages of this process to reach at pre-ovulatory stage. However most of the follicles degenerate before ovulation (Gulliver, 2014). Gonadotropins from the pituitary (FSH and LH) controls ovarian follicular development from late preantral to preovulatory stages (Campbell *et al.*, 2007).

A key component determining the multiparous features of sheep is follicle growth, and increasing the sheep's multiparous capacity will significantly increase the effectiveness of reproduction and production of animals (Wang *et al.*, 2022). The follicle, which serves as the ovary's fundamental functional unit (Bebbere *et al.*, 2020) is made up of oocytes surrounded with the theca and granulosa cells and biological processes such as development, maturation, and degeneration evolve alternatively throughout the estrous cycle (Huang *et al.*, 2016). Granulosa cells and oocytes interact during the early phases of follicular developments and the specificity of steroid hormones released by oocytes and intra-ovarian regulators regulate follicular growth. The balance of granulosa cell proliferation and apoptosis also plays an important role in follicle dominance selection (Orisaka *et al.*, 2009; Liu *et al.*, 2013).

When formed, follicles are composed of the oocyte (female germ cell) surrounded by a single layer granulosa cells. After growth of follicles begins, development of follicles is identified by the recruitment and expansions of other supporting cells called as theca cells, as well as proliferation of the granulosa cells (Gulliver, 2014). The oocyte develops and matures further and granulosa and theca cells differentiated, so they interact with the hypothalamus, pituitary, and reproductive tract and react to endocrine factors regulating ovulation which is the final maturation of oocyte and luteinisation for subsequent p<sub>4</sub> production to maintain early pregnancy (Gulliver, 2014).

Since most of the ovarian follicles go through this cascade rather than the pathway of ovulation, mechanisms regulating degeneration of the follicles are another crucial part of this development. It is well known that follicles can become atretic at any stage of development, from primordial follicles all the way up to follicles that reached pre-ovulatory size, but fail to ovulate and atretic (Matsuda *et al.*, 2012; Juengel *et al.*, 2018).

There are two phases of follicular development: the first phase is the gonadotropins (FSH and LH) independent phase represented by the growth of follicle from the primordial to large antral follicle, and the second phase is the gonadotropin dependent phase (figure 4) where the follicle reach the pre-ovulatory stage and will ovulate, if adequate frequency and amplitude of LH pulse is there (Webb *et al.*, 2004). The difference in the number of follicles that ewes ovulate during their reproductive life is insignificant relative to the number of follicles she is born with (Aerts and Bols, 2010). The follicle's primary fate thereafter appears to be atresia or degeneration rather than ovulation. This appears to follow a process of selection to guarantee that only follicles with oocytes that increase the likelihood of fertilization ovulate (Rosales-Torres *et al.*, 2012).

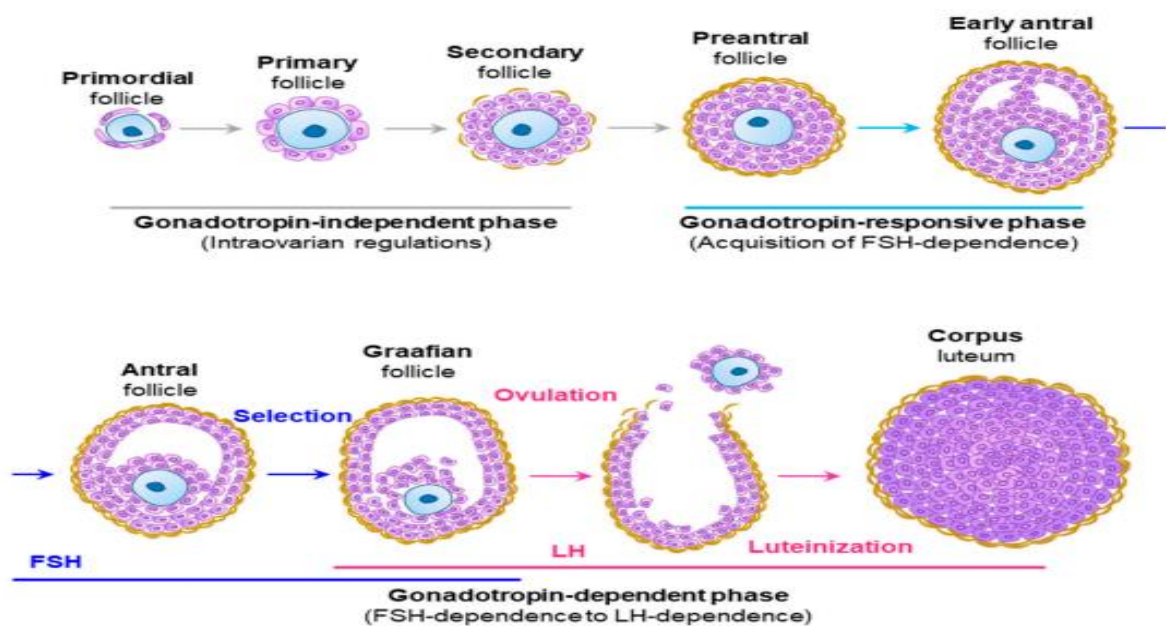


Figure 4: diagram showing the follicular development and subsequent ovulation. Adapted from Lee *et al.* (2021).

### ***Follicular wave***

Follicular wave refers to the synchronized growth of a number of tiny antral follicles from which a single follicle is chosen to become the dominant one, while the remaining follicles go through atresia (Garcia-Guerra *et al.*, 2018). The pattern of follicular wave in sheep is manifested by the development of 3 to 4 (Rosales-Torres *et al.*, 2012) follicular waves during each cycle.

FSH levels are elevated during the early stages of antral follicle formation, and as the follicles develop, they produce inhibin and estradiol, which in turn reduce FSH release (Medan *et al.*, 2005). Although the exact mechanisms governing follicular dominance and deviation in small ruminants are still unknown (Medan *et al.*, 2005; Bartlewski *et al.*, 2011). Generally, one or more follicles are chosen to dominate and acquire LH receptors in granulosa cells to continue their growth at around 1.5 days after emergence (Evans *et al.*, 2000). The development of antral-follicle is categorized into 3 phases: recruitment, selection and dominance phase (Rosales-Torres *et al.*, 2012).

Cyclic recruitment is the stimulation of the growth of cohort of large antral follicles in response to a rise in FSH concentration (McGee and Hsueh, 2000; Webb *et al.*, 2004). Every wave of follicular growth is pioneered by a brief peak of FSH which lasts for 1 to 2 days (Mihm *et al.*, 2000). The wave of growth is prevented from occurring or is postponed, if this peak is experimentally suppressed (Quirk *et al.*, 2004).

In each wave, one or more recruited follicles are chosen to continue growing through a process known as follicular selection (Rosales-Torres *et al.*, 2012). The ability to produce E<sub>2</sub> and respond to gonadotropins has been mentioned as crucial traits that the follicles must possess in order to be chosen and avoid degeneration (Fortune *et al.*, 2001). To cause the pre-ovulatory surge of LH and induce ovulation, the chosen follicles should be able to release significant levels of E<sub>2</sub> (Fortune *et al.*, 2001).

According to the species, dominance is the process by which one or more follicles develop quickly in the endocrine environment where the growth of other follicles is hindered. The presence of molecular machinery which allows it to release large amount of E<sub>2</sub> and its ability to respond to low levels of FSH are the reasons that a given follicle is being dominant (Rosales-Torres *et al.*, 2012).

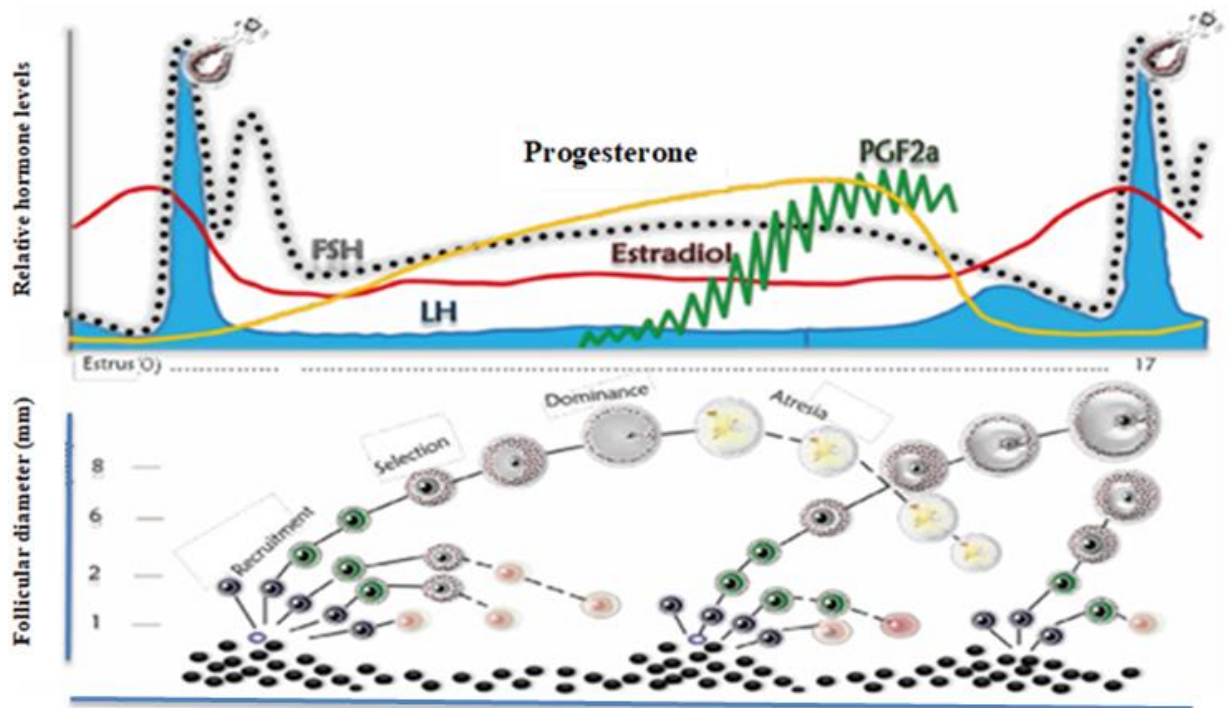


Figure 5: Diagrammatic illustration of follicular development and hormone levels in ewes during the estrous cycle. Adapted from Ramírez-Reyes *et al.* (2021)

## 2.2. Increasing Ovulation Rate

One of the most important component of estrous cycle, ovulation is necessary for successful conception and subsequent pregnancy. The ewe's ova have a fertilizable life span of 10 to 25 hours. During ovulation, a ewe's ovary generates and releases one or more eggs, which is controlled by a reproductive hormone feedback loop. Due to the combined effect of genetic and environmental factors, ovulation rate of sheep can vary significantly between and within breeds. Ovulation rate determines the maximum number of lambs that may be born (Hafez *et al.*, 2000)

The complex exchange of endocrine signals between the pituitary and the ovary, as well as the targeted exchange of intra-ovarian hormones between the oocyte and the surrounding somatic cells, regulate the physiological rate of ovulation in mammals (McNatty *et al.*, 2003). The Ovulation rates in most domesticated sheep breeds range from 1 to 2. But in certain breeds, the ovulation rate is close to or even higher than 3 (Banchero *et al.*, 2021).

A ewe's genetic potential determines its maximum ovulation rate; nevertheless, some environmental circumstances, such as age, diet, season (photoperiod), and socio-sexual cues, might affect an individual's ability to achieve this upper limit (Martin *et al.*, 2004). Among these environmental factors, modifying the diet of ewes offers an easy and cheap way to way to boost

ovulation rate (Martin and Kadokawa, 2006), which reduce the needs for the use of expensive chemicals or hormonal treatments (Martin *et al.*, 2004).

In sheep, flushing, or increasing feeding before mating, can raise ovulation rates (Viñoles *et al.*, 2009). Formerly, prolonged feeding schedules that lasted several weeks were the only way to flush (Gulliver, 2014). However, short-term supplementation for only a few days few days have shown similar increases in ovulation rate. These short-term treatments need to be administered at a certain point in the estrous cycle in order to maximise the rate of ovulation (Scaramuzzi *et al.*, 2006).

Induction of ovulation rate by nutrition provides a short-term "Clean, Green, and Ethical" management approach to boost a sheep flock's reproductive productivity (Martin and Kadokawa, 2006). Though non-nutritional methods like genetic selection may also be clean, green, and ethical approaches they require long term investment. Additionally, alternative non-nutritional ways of boosting ovulation rate, like the use of pharmaceuticals such as Fecundin®, now marketed as ovastim®, *polyandroalbumin 600mg/L*, *virbac Australia* might not meet future market demands and end up adding to the system's expenses (Martin *et al.*, 2004). Therefore, nutritional approaches offer a useful, affordable, and consumer-friendly way to raise the ovulation rate in sheep flocks, especially if they can be applied timeframe (Gulliver, 2014).

### **2.3. Sheep Breeds of Ethiopia**

Ethiopia is a home for at least 9 breeds and 14 traditional sheep populations (Gizaw *et al.*, 2007). The sheep types could be categorized into four groups (sub-alpine short-fat-tailed, highland long-fat-tailed, lowland fat-rumped and lowland thin-tailed) based on their ecological distribution, tail types (fat-tail versus thin-tail), tail form/shape and fibre type (Gizaw *et al.*, 2008). Both within the major sheep groupings and across the different species of sheep, there is a significant degree of morphological and ecological variation. Additionally, there is a significant correlation between sheep production systems, ethnic groups, and types. To create logical conservation-based development programmes, it is crucial to evaluate the genetic distinctiveness of the historic sheep kinds. An extension of this work is the molecular genetic evaluation of the population structure (Gizaw *et al.*, 2008).

The current breeds in Ethiopia are suited to the environmental constraints of the nation, such as limited feed supplies and disease outbreaks (Adimasu *et al.*, 2019). Ethiopia is seeing a rise in

the demand for animal products (small ruminants), particularly mutton, from both internal and international markets (Adimasu *et al.*, 2019). Currently, native sheep output is insufficient to supply this need. It seems that raising sheep productivity results in wealth and raises smallholder farmers' quality of living. Similarly the nation's strong demand for meat must be met (Lakew *et al.*, 2014).

Table 1: Indigenous sheep types of Ethiopia.

Breed group	Breed	Population	Tail type/shap	Fiber type
Short-fat-tailed	Simian	Simian	Fatty and short	Fleece
	Short-fat-tailed	Sekota, Farta,Tikur, Wollo,Menz	Fatty and short	Fleece
Washera	Washera	Washera	Fatty and short	Hair
Thin-tailed	Gumz	Gumz	Thin and long	Hair
Long-fat-tailed	Horro	Horro	Fatty and long	Hair
Bonga	Bonga	Bonga	Fatty and long	Hair
Fat-rumped sheep	Afar	Afar	Fat rump/fat tail hair	Hair
	BHS	BHS	Fat rump/tiny tail	Hair

BHS=Blackhead Somali; **Source:** Gizaw *et al.* (2008)

### 2.3.1. Description of Menz breed sheep

The Menz breed is a small sheep breed with a coarse wool fleece and a wither height of about 60 cm (Weldeyesus, 2020). Adult animals typically weigh between 22 kg for females and 25 kg for males. This breed has acclimated itself to high altitudes and cold temperatures. They have a short and fat tail, and are medium-sized with semi-pendant ears, but they can also be short or rudimentary (12%). White, black and plain brown are the dominant colors of these sheep breeds. Males typically have horns, but females typically don't. Menz sheep are kept in small flocks of no more than 30 animals. Pastoralists primarily exploit three products: meat, dung, and fleece.

The Menz breed of sheep is the only one raised in Ethiopia that produces wool; all other kinds yield coarse hairy fibres. The central Ethiopian highlands, in the Menz district, are home to the traditional Menz sheep rearing area. The fleece from this breed is used to make locally produced handicrafts, giving local artisans a source of income. Menz sheep can survive on low-quality roughages because they are acclimated to the harsh temperature of the area (Gizaw *et al.*, 2007).

Yet one of the things restricting the profitability is the hostile agroclimate, which hinders their growth (Gelaye *et al.*, 2017).

#### **2.4. Role of Nutrition on Reproduction**

There are established definitions for how nutrition affects ovulation rate. Three effects are considered: the static, dynamic, and immediate/acute (Somchit-Assavacheep, 2011). The term static effect describes how ovulation rates in heavy-weight ewes are often higher than in lighter animals. The dynamic effect is characterised as an increase in live weight and body condition score just prior to the estrus period and mating that causes an increase in the ovulation rate. Short-term supplementation with a high energy, high protein meal causes an increase in ovulation rate without changing live weight or body condition score; this is known as the intermediate/acute impact (Viñoles, 2003).

Nutrition has both acute and static impacts on ovarian folliculogenesis (Viñoles *et al.*, 2005), with glucose, leptin, insulin-like growth factor-1 (IGF-I), and insulin acting directly on the ovary (Hernández-Marín *et al.*, 2022). As follicles contain glucose transporter proteins and particular receptors, the current theory regarding the immediate effect of nutrition on folliculogenesis holds that metabolites and metabolic hormones act as signals directly on follicles to regulate folliculogenesis (Scaramuzzi *et al.*, 2006; Ying *et al.*, 2011).

The hypothalamo-pituitary-ovarian axis negative feedback loops and gonadotropins, in particular FSH (figure 6), are crucial for regulating the follicular response to dietary stimulation (Somchit-Assavacheep, 2011). Changes in ovarian function may be linked to nutritionally driven variations in a variety of metabolic hormones (Somchit-Assavacheep, 2011). Additionally, ovarian steroids have the ability to control the synthesis and activity of these metabolic hormones, creating dynamic positive and negative feedback loops (Webb *et al.*, 2004). Although the primary target of nutritional influence on folliculogenesis is the follicle, the actual mechanism probably involves follicle centred alterations of homeostasis in the negative feedback loops of the hypothalamo-pituitary-ovarian axis. The follicle is the main target of dietary influence on folliculogenesis; however, the true mechanism likely involves follicle centered changes in homeostasis in the hypothalamo-pituitary-ovarian axis negative feedback loops (Somchit-Assavacheep, 2011).

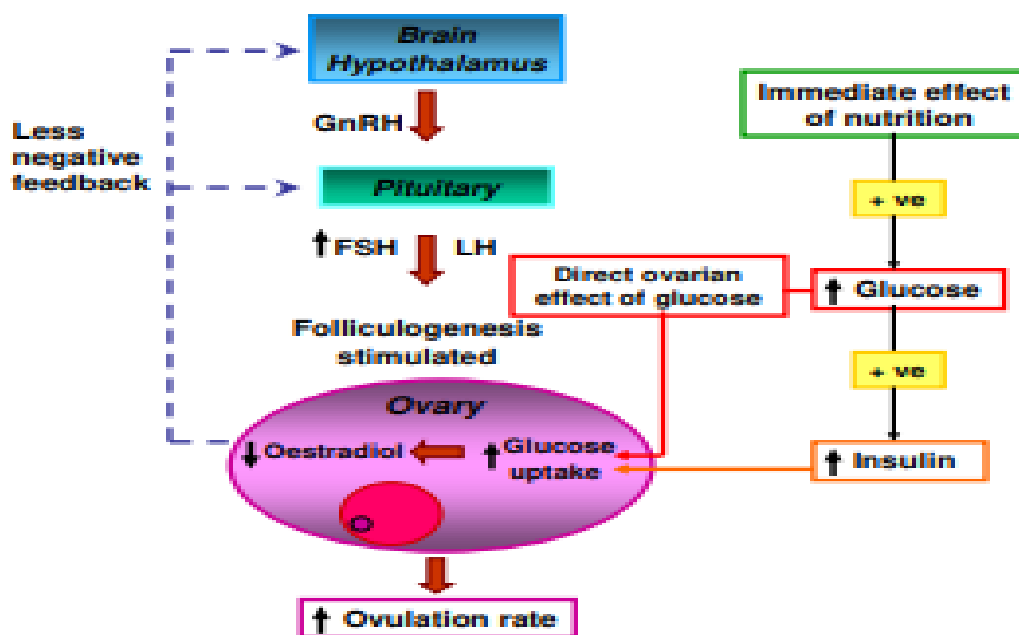


Figure 6: A model to explain the immediate effect of nutrition on the regulation of folliculogenesis and ovulation rate in ewes, involving the negative feedback loops of the hypothalamus-pituitary-ovarian axis. Adapted from Somchit-Assavacheep (2011).

Animal reproduction is significantly impacted by nutrition (Vijayalakshmy *et al.*, 2020). Major physiological processes of reproduction, such as ovulation rate and prolificacy, are influenced by nutrition during the formation of ovarian follicles, especially in large-scale sheep production systems (Martin *et al.*, 2004).

It has long been recognised that the relationship between diet and reproduction has significant effects on ewes' ability to reproduce. Multiple levels of reproductive function are modulated by nutrition through the action of circulating metabolic hormones, including growth hormone, glucose, insulin, and IGF-I. Because these hormones govern the action of major reproductive hormones including gonadotropins and steroids in follicles, they play crucial roles in the control of follicle growth and are likely mediators of the effects of dietary intake on ovulation rate (Somchit-Assavacheep, 2011).

## 2.5. The Metabolic Effect of Nutritional Supplements in Ewe

### 2.5.1. Glucose and insulin

Numerous research findings point to insulin's critical role as a signal that modifies the impact of acute alterations in dietary intake on follicle dynamics (Armstrong *et al.*, 2003). Insulin levels in the blood fluctuate throughout the estrous cycle and are influenced by food consumption and

substantial increase in insulin concentrations is linked to ovulation (Armstrong *et al.*, 2003). In female sheep, feeding 500g of lupin daily, as two meals of 250g in the morning and 250g in the evening raised the concentrations of plasma glucose and insulin (Scaramuzzi *et al.*, 2006).

#### 2.5.2. *Leptin*

Adipocytes are the primary producers of leptin, and mounting data suggests that this hormone may serve as a signal that connects reproductive function and nutritional status (Spicer, 2001). Plasma leptin concentrations were raised by feeding of 500g of lupin twice a day, 250g in the morning and 250g in the evening, as well as by intravenous glucose infusion (Scaramuzzi *et al.*, 2006). The concentration of leptin in the blood informs the follicle of the long-term consequences of weight gain (dynamic and static effects) as well as the immediate effects of dietary supplements that do not alter body weight (Scaramuzzi *et al.*, 2006).

#### 2.5.3. *Insulin like growth factor*

There is an evidence of dietary-induced variations in the IGF-I system circulation concentrations (Armstrong *et al.*, 2003) and there is a positive correlation between circulating IGF-I concentrations and feeding frequency (Rausch *et al.*, 2002).

#### 2.5.4. *The effect of nutritional supplements on follicular growth*

A 3 or 5 day glucose infusion increased the number of large follicles while having no effect on the number of small and medium-sized follicles, which indicates how folliculogenesis is triggered (Muñoz-Gutiérrez *et al.*, 2005). Large follicles were more frequently produced by lupin feeding, although a lesser extent than glucose infusion. The primary effect of lupin feeding was on medium-sized follicles, whose numbers were roughly doubled as a result of lupin feeding (Muñoz-Gutiérrez *et al.*, 2005). A systemic dose of 1  $\mu\text{g}\cdot\text{h}^{-1}$  of leptin infusion resulted in a rise in the number of large follicles  $> 3.5$  mm, whereas the number of medium-sized follicles (1–3.5 mm) remained unchanged (Kendall *et al.*, 2004). These findings imply that the glucose-insulin and leptin systems are not the only mechanisms via which lupin grain supplementation affects folliculogenesis.

## 2.6. **Flushing**

Flushing in general refers to the short-term and targeted feeding technique before, during or after mating (Shad *et al.*, 2011). Commercial goat and sheep producers have long used nutritional

management techniques to boost ovulation, conception, and implantation rates, which explains the rise in the number of kids and lambs produced. The basis for flushing is that, nutrition and reproduction have a unique relationship and this association has far-reaching consequences for the ability of sheep to reproduce. Therefore, receiving the right nutrients at the right time is crucial for successful reproduction, which rises the profitability of sheep and goats production system (Assan, 2022).

The time of flushing is very critical to its effectiveness. For the goal of increasing ovulation, conception, and embryo implantation rates, it should be intended to temporarily but deliberately elevate the nutritional plane just around breeding season. In addition flushing is important in rising the percentage of estrus-exhibiting females. Flushing is again a crucial management strategy to enhance reproductive efficiency in ewes, as their nutritional needs vary throughout the reproductive cycle (Assan, 2022).

Nowadays, feeding ewes for longer times like 2 or 3 weeks is not necessary, and supplementing for about 9 days prior to mating is sufficient to encourage an increase in the rate of ovulation (Viñoles, 2003). These days, the ewe's effective time for nutritional therapy has been drastically shortened targeting a very critical periods of the estrous cycle. This significant and scientific advancement reduces the cost of supplemental feeding which is crucial for farm economics. For the effectiveness of this short-term nutritional supplementation of ewes, estrus should be synchronized since there is a key period throughout the estrous cycle when short-term supplementation enhances ovulation rate (Assan, 2022). Many researchers have attested to the efficacy of brief dietary supplementation in elevating the rate of ovulation in ewes (Vinoles, 2003; Viñoles *et al.*, 2005; Scaramuzzi *et al.*, 2006). Consequently, focus feeding has been developed and now developing to enhance small ruminants' reproductive capabilities (Habibizad *et al.*, 2015).

### 3. MATERIALS AND METHODS

#### 3.1. Study Area

This study was conducted in Bishoftu town, Ethiopia, which is located in East Shewa zone of Oromia regional state, 47 km southeast of the capital city Addis Ababa. The absolute location of the area is 8°45'N latitude and 38°59'E longitude (Tibebu *et al.*, 2021). Bishoftu experiences an average annual rainfall of 1150 mm, with 84% occurring during the long rainy season from June to September, and the remaining rainfall during the short rainy season from March to May. The mean annual minimum and maximum temperatures in Bishoftu are 8.5 and 30.7 °C, respectively, and the average relative humidity is 61.3% (Birhanu *et al.*, 2017).

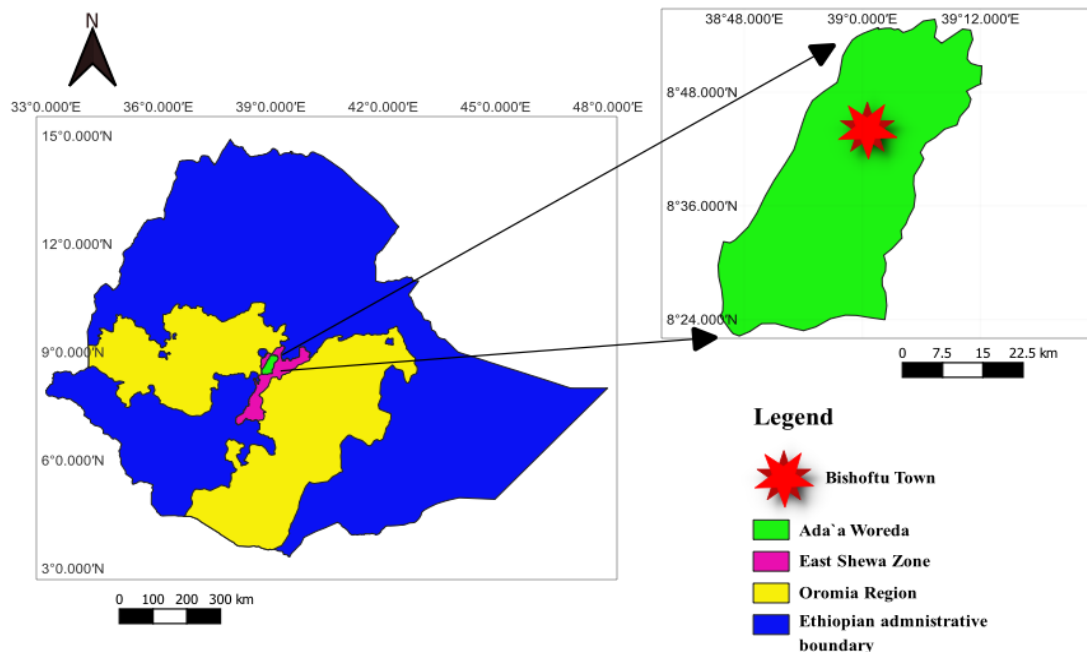


Figure 7: Map of Bishoftu town (the study area).

#### 3.2. Experimental Animals

The experiment was done on healthy, normally cyclic and non-pregnant Menz breed ewes. A total of 24 mature Menz breed ewes were purchased from the local Menz market for this purpose. All were dry ewes with body weight ranging from 20.5 to 24 kg and body condition score between 2.5 and 3 based on 1-5 scale of measurement. All the ewes were at active reproductive age (3-5 years) and at least a parity of one. The ages of animals were determined by dentition and information from the owners during purchasing.

### 3.3. Experimental Design and Animal Management

An experimental study design was conducted on a total of 24 Menz breed ewes. Experimental animals were ear-tagged for the easy of identification and management system. Animals were quarantined for one month before the start of the experiment. This period allowed them to adapt to the new environment and provided an opportunity to observe their health status. Additionally, it was important to verify whether the ewes exhibited normal cyclic behaviour for at least one complete cycle. During this period animals were vaccinated against sheep pox and ovine pasteurellosis. They are also dewormed with albendazole (*Ashialben®300, Ashish Life Science Pvt. Limited, Mumbai-India*) against internal parasites and sprayed with acaricides (*Diazinon, Vetazinon 60% EC, Adamitulu-Ethiopia*) against external parasites.

Before the beginning of the experiment, all the animals were checked for reproductive tract abnormality and the presence of pregnancy using ultrasonography and only non-pregnant ewes with normal reproductive tract were used for the experiment. Ovarian cyclicity and fertility were checked before the start of the experiment using ultrasonography and observation for at least one complete estrous cycle.

The experimental animals were assigned into three groups using randomized block design: two treatment groups (T1 and T2) and one control group (C). Each group consisted of 8 ewes. The ewes had ad libitum access to water and grass hay before and during the experiment. In addition, the ewes were allowed to graze freely for 8 hours each day. Treatment group T1 (T1; n=8 ewes) consisted of ewes supplemented with 500g of lupin grain per day per head (250g in the morning and 250g in the evening). Treatment group T2 (T2; n=8 ewes) comprised ewes supplemented with 500g of barley grain per day per head (250g in the morning and 250g in the evening). The third group (C; n=8 ewes) is a control group without any supplementation except the common basal diet (grass hay). The total duration of nutrient supplementation was 9 consecutive days during the late luteal phase of estrous cycle.

#### 3.3.1. Estrus synchronization, detection and mating

Before the start of the experiment, ewes were synchronized using prostaglandin (PGF<sub>2</sub>α) (*Synchromate®, Cloprostenol sodium, Warburg, Germany*) given 9 days apart targeting the critical late luteal phase of the estrous cycle. Supplementation of the selected feed was started seven days after the first signs of estrus following the second injection of PGF<sub>2</sub>α and continued for 9 consecutive days (Figure 8). Ewes were observed for manifestation of behavioural estrus

after 9 days of nutritional supplementation. Estrus was observed for one hour three times a day (12:00 to 7:00am, 1:00-2:00pm, and 6:00-7:00pm) for two consecutive days (48 hours) using a vasectomised ram.

Ewes were considered as in estrus when they “stand to be mounted” by vasectomised rams. Other signs were also recorded and considered to support the decision. The onset and duration of estrus were recorded for later analysis. All ewes in standing estrus were allowed to be mated naturally by Dorper breed rams. Pregnancy was first checked on day 35 using Pregton (Renco, Minneapolis, USA) and ten days later confirmed for twinning using a B-mode ultrasound with linear array trans-rectal transducer (DP 50vet, China with a 7.5 MHz linear array rectal transducer).

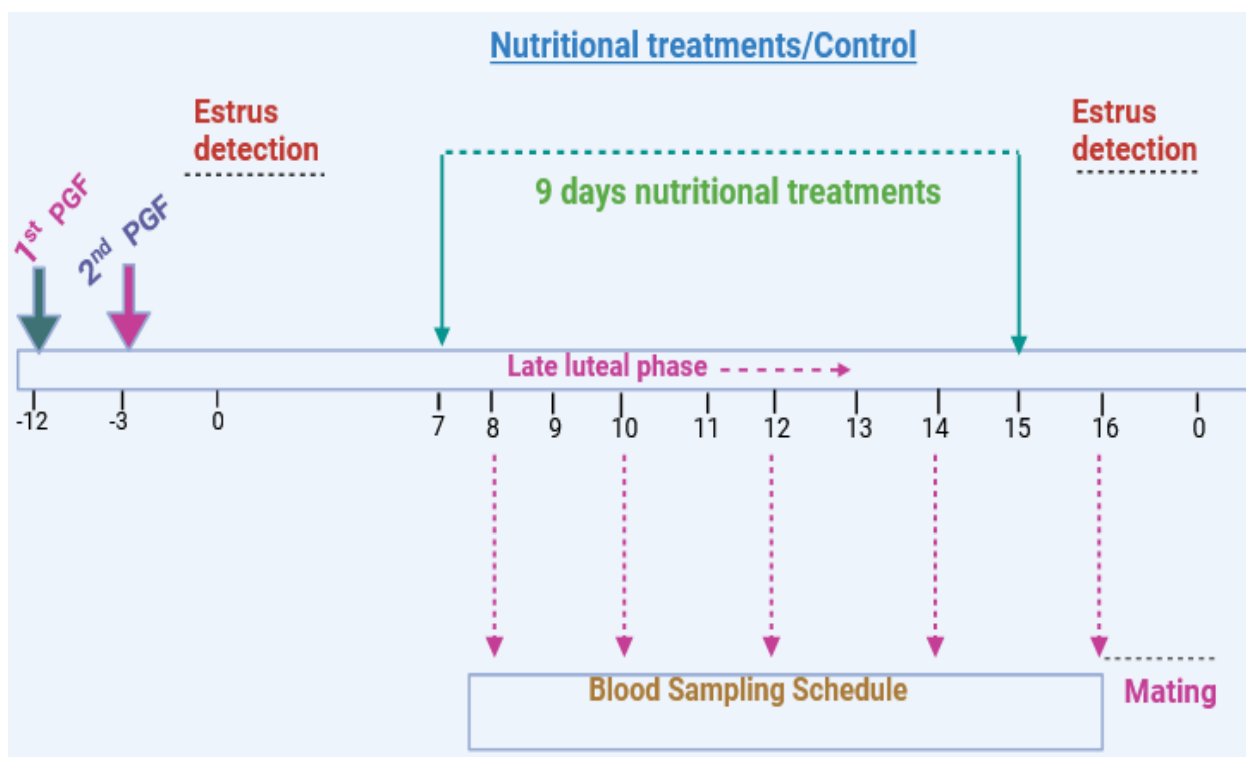


Figure 8: Schematic representation of estrus synchronization, blood sampling and targeted time for short-term nutritional supplementation schedule.

### 3.3.2. Ultrasound examination

Trans-rectal ultrasound examinations were conducted using a real time B-mode scanner with 7.5 MHz linear array rectal transducer. For ultrasonography, ewes were handled in a quadruped position, with the abdominal wall compressed upwards, to facilitate the visualization of the uterus (Viñoles *et al.*, 2004; Brogni and de Souza, 2021). The faecal pellet was removed and ultrasound gel was inserted into the rectum to act as a coupling medium between the rectal wall

and the transducer. Pregnancy was checked at day 35 using Pregtone (RENCO, MINNEAPOLIS, MN.5540, USA) and after 45 days they were checked for confirmation of pregnancy, twinning or presence of prolificacy using Ultrasound (Mindray DP.50vet, China with a 7.5 MHz linear array rectal transducer).

### *3.3.3. Blood sampling*

Blood (5ml) sample was collected in an EDTA vacutainer tube from all experimental animals via jugular venepuncture every 48 hours during the 9-day feeding period to monitor plasma glucose levels. The blood samples were then centrifuged at 4000 RPM for 10 minutes, and the plasma was transferred to a cryovial, labeled, and stored at -20 °C until further processing.

### *3.3.4. Plasma glucose analyses*

Glucose was analysed in all plasma samples, by Atellica-CH Analyzer using the glucose hexokinase method (Atellica CH Glucose Hexokinase\_3 (GluH\_3); at Wudassie Diagnostic laboratory Ltd, Addis Ababa, Ethiopia). The Atellica CH Glucose Hexokinase\_3 (GluH\_3) assay employs a two-component reagent as its basic testing premise. Reagent 1, which comprises the buffer, ATP, and NAD, is mixed with the sample. Reagent 1 is used to take absorbance values of the sample, which are then used to adjust for any interfering chemicals. The addition of Reagent 2 starts the conversion of glucose and the growth of absorbance at 340/410 nm.

Reagent 1 and Reagent 2's absorbance differences are related to the glucose concentration. A dilution cuvette was filled with 200µL of Atellica CH diluent and 50µL of primary material. Next, 53.3µL of special reagent water and 26.7µL of Reagent 1 were poured into a reaction cuvette. Next, 3.4µL of the material that had been pre-diluted was put into a reaction cuvette. After adding the sample, the absorbance was measured. A reaction cuvette was filled with 16µL of Reagent 2, which was then mixed and incubated at 37°C. After adding Reagent 2, the absorbance was finally measured, and the outcome was either reported or shown on the computer screen.

## **3.4. Data Management and Analysis**

The body weight, onset of estrus, duration of estrus, mean concentration of plasma glucose, pregnancy rate, prolificacy rate, and other relevant data were coded, entered, and managed using Microsoft Office Excel (2010). The data were then analyzed using STATA statistical software release 14. Body weight and plasma glucose concentrations were analyzed using repeated

measures ANOVA. Onset of estrus, duration of estrus, and prolificacy rate were analyzed using one-way ANOVA. The pregnancy rate of ewes in each treatment group was compared using the chi-squared test of independence ( $X^2$ ).

### **3.5. Ethical Clearance**

All experimental procedures and activities undertaken in this study were conducted in strict accordance with the rules and guidelines of Ethics and Animal Welfare. Prior to implementation, the research protocol underwent a rigorous review process by the Institutional Animal Care and Use Committee (IACUC) of Addis Ababa University College of Veterinary Medicine and Agriculture Bishoftu, Ethiopia. The committee carefully evaluated the proposed procedures to ensure compliance with ethical standards and animal welfare considerations. Approval from the IACUC was obtained (Annex, 6) affirming that the study design and procedures met the necessary ethical guidelines. This diligent scrutiny and approval process provided assurance that all necessary precautions were taken to prioritize the well-being and ethical treatment of the animals involved in the study.

## 4. RESULTS

### 4.1. Body Weight Change

The mean body weights measured at the beginning and end of the short-term nutritional supplementation of ewes in different experimental groups are presented below in table 2. There was no significant difference ( $P > 0.05$ ) in the mean body weight of the ewes at the beginning of the experiment (IMBW) which confirmed that the experimental animals were evenly distributed across all the groups. Furthermore, the mean body weight did not differ after feeding (FMBW) and between experimental groups (BGBW) within the experimental period ( $P > 0.05$ ) suggesting that changes in weight were not influenced by the short-term nutritional supplementation in this study ( $P > 0.05$ ).

Table 2: Body weight (kg) changes after short-term nutritional supplementation of Menz breed ewes.

Weight source (kg)	Treatment groups			SD	P-value
	T1	T2	C		
IMBW	22.56	22.19	22.25	1.19	0.8117
FMBW	22.63	22.38	22.44	1.15	0.8909
BGBW	22.59	22.34	22.31	0.96	<b>0.7631</b>

*IMBW=Initial Mean Body Weight; FMBW= Final Mean Body Weight; BGBW= Between Group Body Weight; SD=Standard Deviation*

### 4.2. Onset and Duration of Estrus

The mean time (hours) to the onset of estrus (Table 3) varied significantly ( $P < 0.05$ ) between groups and was shorter in the treatment groups T1 and T2 (supplemented groups) than that of the control group (C) where treatment groups T1 and T2 accelerate the ewes to come into estrus earlier than the non-supplemented control group.

Similarly, the mean time to duration of estrus was statistically significant ( $P < 0.05$ ) between groups T1, T2 and C and where shorter estrus duration was recorded in non-supplemented control group C ( $29.13 \pm 1.44$ ) followed by treatment group T2 ( $35.63 \pm 2.71$ ) and T1 ( $41.5 \pm 2.19$ ) as shown below in Table 3. The supplemented groups T1 and T2 render the ewes to stay in estrus for longer time as compared to non-supplemented control group (C).

Table 3: Onset and duration of Estrus (hours) after short-term nutritional supplementation of Menz breed ewes.

<b>Treatment Groups</b>	<b>Onset of Estrus (mean <math>\pm</math> SD)</b>	<b>Duration of estrus (mean<math>\pm</math>SD)</b>
T1	$29.25 \pm 2.88$	$41.5 \pm 2.19$
T2	$31.5 \pm 2.95$	$35.63 \pm 2.71$
C	$42 \pm 1.96$	$29.13 \pm 1.44$
P-value	0.0057**	0.0025**

*SD=Standard Deviation; \*\*=Significant at ( $P < 0.001$ )*

### **4.3. Blood Plasma Glucose Concentration**

The level of plasma glucose varied significantly ( $P < 0.05$ ) across the groups of ewes. Short-term supplementation of ewes with high protein and energy diet resulted in increased plasma glucose level compared to the non-supplemented control group (figure 9). Furthermore, blood plasma concentration of glucose was higher in lupin supplemented group of ewes compared to ewes supplemented with barley grain. The variation between groups becomes more significant with duration of supplementation ( $P < 0.05$ ) or in other words; there were significant effect of time on the plasma glucose level ewes in the treatment groups.

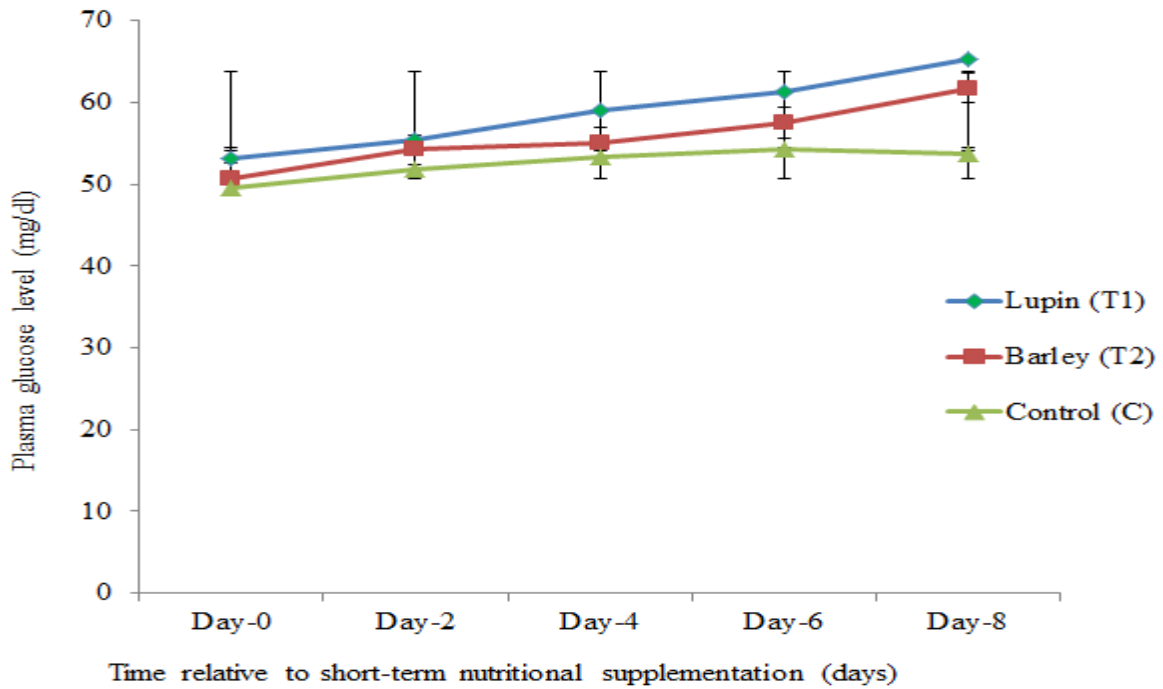


Figure 9: The mean plasma glucose concentrations in Menz ewes fed hay (Control: n=8), hay supplemented with 500g lupin grain (Lupin: n=8) and hay supplemented with 500g of barley grain (Barley: n=8).

Plasma glucose concentrations were varied significantly ( $P < 0.05$ ) between the treatment groups by days of feeding starting from the third sampling day (Day 4 of feeding) (Table 6).

Table 4: The plasma glucose concentration level (mg/dl) in relation to feeding time and treatment types in Menz breed ewes.

<b>Treatment Groups</b>				
<b>Days</b>	<b>T1(mean ± SD)</b>	<b>T2 (mean ± SD)</b>	<b>C (mean ± SD)</b>	<b>P-value</b>
0	53.13 ± 4.22	50.75 ± 2.38	49.63±4.47	0.1960
2	55.38 ± 2.92	54.25 ± 1.91	51.88 ± 7.08	0.3126
4	59± 3.55	55.13 ± 2.30	53.38 ± 1.30	0.008**
6	61.38 ± 5.15	57.5 ± 2.44	54.25 ± 2.19	0.0024***
8	65.63 ± 3.89	61.75 ± 2.66	53.75 ± 3.11	0.0000***
<b>Total</b>				<b>0.0000***</b>

*SD=Standard Deviation; \*\*=Significant at ( $P < 0.001$ )*

#### 4.4. Pregnancy Rate

The pregnancy rate of ewes were varied significantly ( $P < 0.05$ ) between the treatment groups T1 (lupin fed group), T2 (barley fed group) and the non-supplemented control groups with the rate of 100%, 87.5% and 50% respectively.

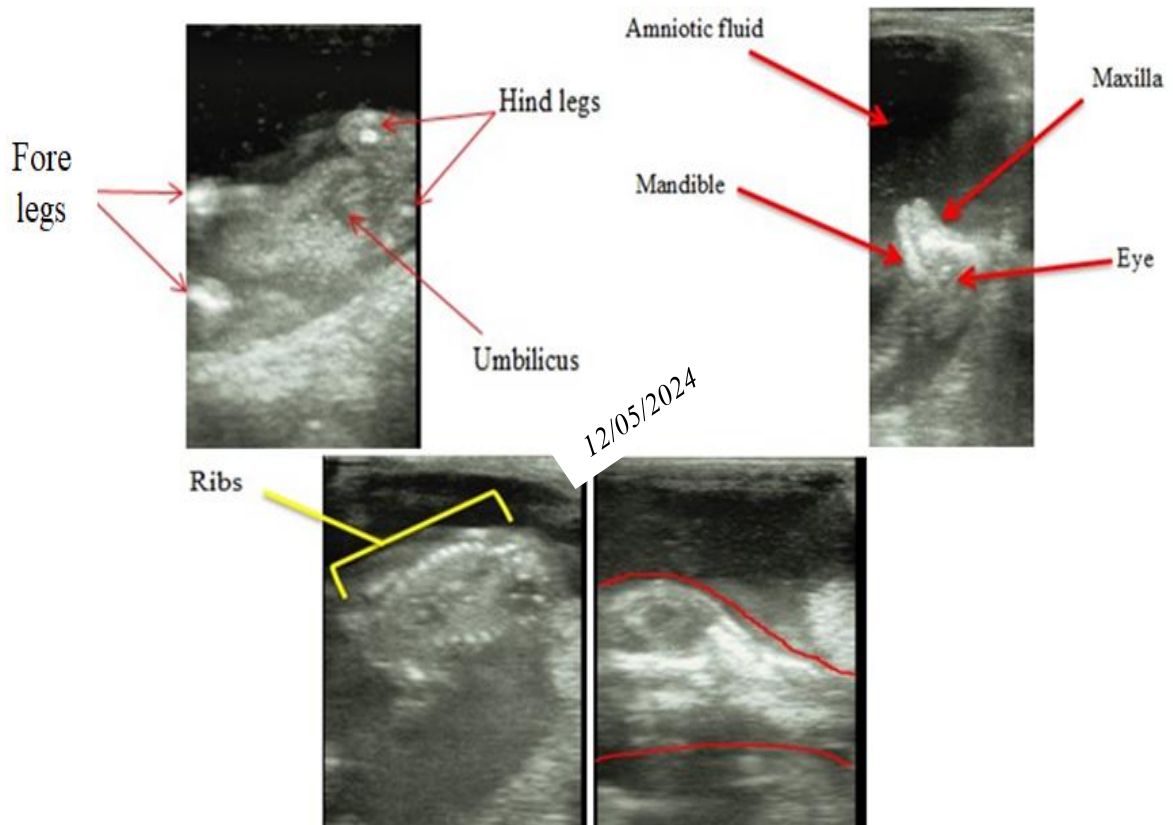


Figure 10: Ultrasound images taken during pregnancy diagnosis in Menz breed ewes after short-term nutritional supplementation.

#### 4.5. Prolificacy Rate

Ewes in each experimental group were routinely diagnosed for the presence of twinning or triple pregnancy or prolificacy by using trans-rectal ultrasound 45 days after mating. As the result suggested, significantly higher ( $P < 0.05$ ) twinning or prolificacy rate was observed in lupin fed group (T1; 50%) followed bay barley fed (T2; 13%) and control groups (C; 0%) of ewes.

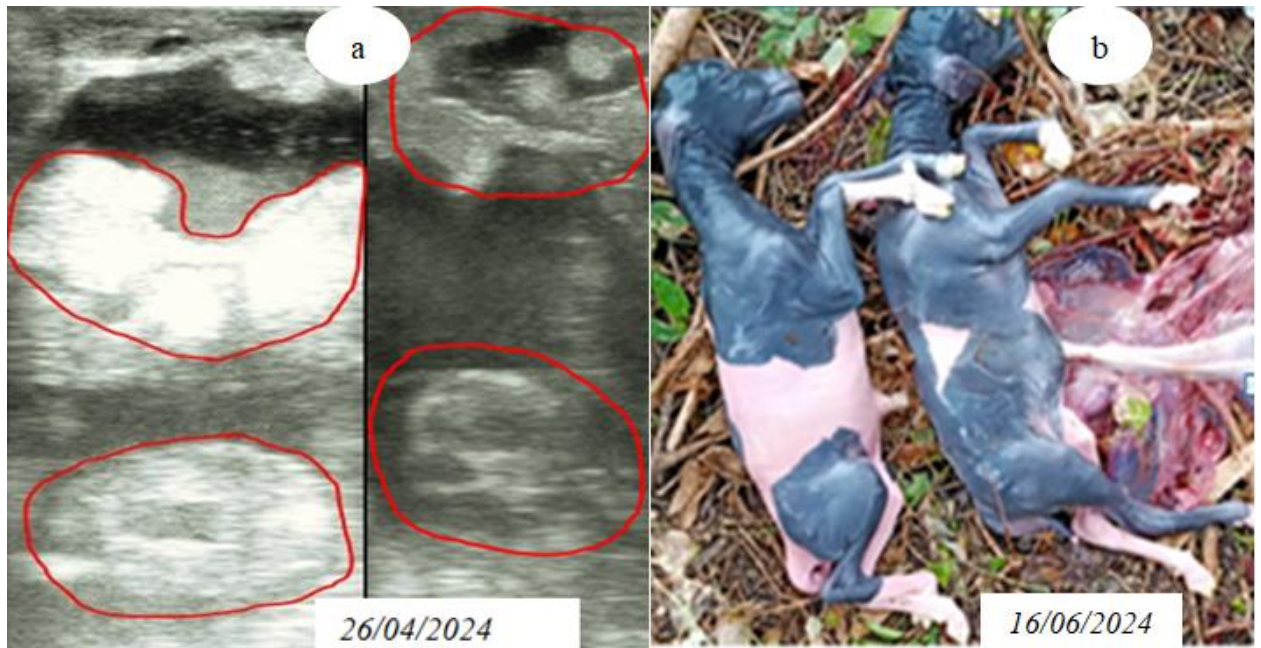


Figure 11: Ultrasonography images (a) and post-mortem examination of a suddenly died ewe (b) showing twin pregnancy in Menz ewes supplemented with lupin grain for 9 consecutive days at the late luteal phase of estrous cycle.

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## 5. DISCUSSION

The hypothesis of the present study was accepted, suggesting that short-term supplementation of a high protein and energy diet during the late luteal phase of estrous cycle would improve the reproductive performance of ewes. Results obtained in this study indicated that short-term supplementation for only 9 days during the late luteal phase of estrous cycle leads to significant changes in the metabolic status (increased plasma glucose level) and reproductive performance (including onset and duration of estrus, pregnancy rate, and prolificacy) of ewes. The study has provided an insight of alternative for improving the reproductive efficiency of sheep by manipulating nutrition, effectively expanding the options for focus feeding and offering strategies for reducing the risks associated with hormonal treatments.

This study suggested that short-term nutrient supplementation of ewes did not lead to statistically significant changes in the mean body weight ( $P > 0.05$ ) with treatment time and between the experimental groups. It is true that improved reproductive performance without a change in the ewes' body weight or body condition score is a characteristic of the immediate effect of feeding (Viñoles, 2003; Scaramuzzi *et al.*, 2006; Somchit *et al.*, 2007). The study also aligns with the result of Somchit *et al.* (2007) who investigate the impact of feeding ewes with 500g of lupin during the late luteal phase of estrous cycle. Similarly, Venturi *et al.* (2016) did not find significant changes in the body weight in Santa Ines breed sheep after short-term supplementation of energy source feed.

Furthermore, this finding regarding body weight changes are in accordance with the results reported by Vinales *et al.* (2005) in Uruguay. Vinales *et al.* conducted a study on Corriedale ewes with short-term supplementation of corn grain and soybean and did not find significant changes in their body weight. The idea that short-term nutritional supplementation may primarily enhance reproductive success without significantly changing body weight in sheep is supported by these consistent results across many investigations.

The neglected effect of nutritional treatment on the body weight of ewes at different time point's evaluated in this study makes it possible to assess the intermediate effect of nutrition on the circulating blood metabolites. It is therefore confirmed that body weight had no bearing on the characteristics that were assessed. Rather, the ovarian follicles are directly affected by the circulating metabolites, which are in turn regulated by the rapid effects of the supplemental meals.

The effect of nutrition on sheep reproduction has been well-described. Acute effects occur when nutritional input is given for brief periods of time (6 to 10 days), increasing ovulation rate without affecting live weight or body condition. Dynamic/static effects occur when supplementation is given for a few weeks, increasing live weight and body condition score and improving fertility (Somchit-Assavacheep, 2011). Therefore; the results in this research undoubtedly support the hypothesis that fertility increase in treatment groups T1 and T2 is the result of the acute/immediate effect of supplemented nutrition acting directly on the follicles.

The mean time to the onset of estrus in this study varied significantly ( $P < 0.05$ ) and was shorter in supplemented groups T1 and T2 than the control group C. This suggests that the higher level of nutrient supplemented to groups T1 and T2 accelerated the ewes coming into heat earlier compared to the non-supplemented control group. In other words, the ewes in the control group took significantly ( $P < 0.05$ ) longer time to estrus behaviour compared to the supplemented groups T1 and T2. The result is in agreement with previous studies by Ayele (2022) who evaluated flushing effect on the body weight, body condition score and the reproductive performance of Doyogena ewes in Doyogena District, Southern Ethiopia and again aligns with Habibzad *et al.* (2015) who conducted their study on the effect of short-term feeding dietary energy on the reproductive performance, serum metabolites and reproductive hormones of ewes in Iran.

The time to commencement of estrus in ewes was not affected by a diet with varied amounts of energy or by using a flushing program in an attempt to boost reproduction, contrary to the current findings (Naqvi *et al.*, 2002). Hernández-Marín *et al.* (2022) also reported contrary result after targeted diet during synchronization of estrus in Pelibuey ewes with varying body conditions. This inconsistency could be due to the difference in breed, age, quality of nutrient provided, and the synchronization protocol used (Boscos *et al.*, 2002). The greater number of pre-ovulatory follicles resulting from the ovarian stimulatory impact of nutrition may be the cause of the early detection of estrus behaviour in ewes fed high protein and energy diet.

This study also revealed that, the mean time to duration of estrus varied significantly ( $P < 0.05$ ) across the experimental groups T1, T2 and the control group (C) where shorter estrus duration was recorded in the control group (C) followed by barley supplemented group (T2) and lupin supplemented group (T1) of ewes where, supplemented groups T1 and T2 render the ewes to stay in estrus for longer time as compared to the control group (C) and this might be due to the effect of supplemented nutrition. This finding is supported by other previous research findings

(Zohara *et al.*, 2014; Daghash *et al.*, 2017; Moghaddam *et al.*, 2021; Ayele, 2022) in that, nutritional supplementation render ewes to stay in estrus for longer time as compared to non-supplemented ewes. The difference in the duration of estrus between the supplemented and non-supplemented groups of ewes might be related to the fact that, nutrient supplementation improve metabolic hormones like glucose, insulin, IGF-I and leptin which intern alter reproductive hormone concentration in such a way that these metabolic hormones reduce estradiol concentration and the reduction in the concentration of estradiol will result in absence of negative feedback mechanism on FSH release from the anterior pituitary.

The nutrients that are absorbed and dispersed throughout the body by the circulatory system are described as blood metabolites (Nurlatifah *et al.*, 2020). This study suggested that short-term supplementation of ewes with high protein and energy diet increases plasma glucose level significantly ( $P < 0.05$ ) and remained higher as compared to non-supplemented control group (C) for the duration of feeding period. In relation to the sampling days, plasma glucose concentration varied more significantly ( $P < 0.05$ ) after the third sampling day between groups with time of feeding. The highest mean concentrations of plasma glucose were recorded in ewes supplemented with lupin grain in each sampling day.

In consistent with the current research finding, many investigators have reported that short-term supplementation ewes improve plasma glucose concentration (Scaramuzzi *et al.*, 2006; Somchit *et al.*, 2007; Ying *et al.*, 2011; Somchit-Assavacheep *et al.*, 2013). The present study also showed very high significant effect of time ( $P = 0.000$ ) as well as time with treatment of nutrition. In line with this finding Viñoles (2003) also investigated an increase in plasma glucose level after intravenous infusion of glucose for five days at late luteal phase of estrous cycle in sheep. This result also disagrees with previous studies by Somchit *et al.* (2007) who conducted a research to evaluate the effect of short- term supplementation of lupin grain for five days at late luteal phase of estrous cycle of Welsh Mountain ewes and Hashem and El-Zarkouny (2014) who also conducted their study the effect short-term supplementation of rumen protected fat for 9 consecutive days during the late luteal phase of the estrous cycle. This discrepancy might be due to the breed of ewes, age, feed quality and duration of supplementation.

Nutrition stimulates the reproductive activity at different levels through the circulating metabolic hormones such as glucose, insulin, insulin like growth factor-I (IGF-I) and leptin. By controlling the activity of critical reproductive hormones like gonadotropins and steroids in follicles, these hormones play significant roles in the regulation of follicle growth and are probably mediators of

the effects of dietary consumption on ovulation rate (Somchit-Assavacheep, 2011). Therefore the pronounced reproductive performance recorded in this research finding related to short-term nutritional supplementation might be due to increased level of these circulating metabolic hormones directly on follicles without significant change on body weight and body condition and the deference between the experimental groups might be due to the quality of supplemented nutrition.

The pregnancy rate was higher in supplemented groups, T1 (100%) and T2 (87.5%) as compared to the non-supplemented control group (50%). Lower pregnancy rate in non-supplemented group of ewes is an indication for their poor status of nutrition as they were not able to achieve their nutrient requirements from the basal diet alone. This is supported by the low concentration of plasma glucose observed compared to the supplemented group of ewes. The higher pregnancy rate in the case of supplemented group of ewes might be due to the effect of nutrition on the blood concentrations of metabolic and reproductive hormones and some of the intra-ovarian changes that take place in response to nutritional stimulation.

In the present study, the pregnancy rate under the treatment group T1 (lupin fed group) was 100% and is in line with the previous study by Farrag (2019) who reported 100% pregnancy rate in Abou-Delik ewes after flushed by 300g barley grain per ewe per day for 21 consecutive days. But this is higher than the report by Ayele (2022) who stated 85.3% and 83.3% pregnancy rate in Doyogena ewes flushed by 400g enset leaf with 500g concentrate and 500g enset leaf with 400g concentrate respectively. Furthermore it is higher than the report by Stanzaki *et al.*, 2022 who stated that the pregnancy rate was 72.72% when Marwari ewes flushed by 300g concentrate supplementation for 21 days and the report by Mostafa and Farghal, 2022 who reported 70% pregnancy rate when Ossimi ewes were flushed with 500g corn per ewe per day for two weeks.

The pregnancy rate in the case of barley supplemented group of ewes (T2) was 87.5% which is slightly agreed with the study by Ayele (2022) who reported 85.3% pregnancy rate in Doyogena ewes flushed by 400g enset leaf with 500g concentrate for 35 days. But a little bit lower than 100% pregnancy rate reported by Farrag (2019) after flushing Abou-Delik ewes by 300g barley grain per ewe per day for 21 consecutive days. However this result is higher than the reports by Stanzaki *et al.*, 2022 and Mostafa and Farghal, 2022 who stated 72.72% and 70% pregnancy rate for Marwari and Ossimi ewes flushed by 300g concentrate supplementation for 21 days and 500g corn per ewe per day for two weeks respectively. From these differences, it is possible to generalize that the conception rate recorded in the current study is very promising in such a way

that by providing high quality diet at the critical period of estrous cycle, it possible to improve the reproductive performance of ewes with minimal expense.

The twinning rate or litter size was significantly ( $P < 0.05$ ) higher (50% or 1.5) in lupin fed group (T1) as compared to barley fed (T2) (13% or 1.13) and control group (C) (0%). The twinning rate found in the case of lupin fed ewes in the present study was highest as compared to previous study reports; 1.08 (Edea, 2008); 1.13 (Mukasa-Mugerwa *et al.*, 2002) and 1.14 (Edea, 2008). Whereas, the twinning rate of found in case of barley supplemented group (1.13) is in consistent with the above mentioned study reports. The higher prolificacy rate in the case of supplemented group in this study indicates the modulatory effect of nutrition on ovulation rate of ewes as described by previous studies (Viñoles, 2009; Banchero *et al.*, 2021; Assan, 2022). A high-protein diet has the potential to accelerate a ewe's ovulation rate when compared to a high-calorie diet (Assan, 2022). The difference between supplemented groups in this research finding reflects the quality of the diet.

Numerous earlier research found that lupin grain supplementation is associated with broad impacts on the biokinetics of glucose and the dietary supply of nutrients that provide energy and the increase in ovulation rate in ewes appears to be mediated through biological pathways linked to the synthesis and utilization of glucose (Somchit *et al.*, 2007). This response has traditionally been explained by the fact that lupins have very low starch content in addition to their high protein content. In ruminants, this results in increased glucose entry rate because it increases the availability of energy substrates, especially acetate, which is a crucial substrate for gluconeogenesis (Somchit *et al.*, 2007). The increased in twinning and conception rate in the case of lupin supplemented group of ewes in this study therefore might be these especial quality of the lupin grain as compared to other source of nutrients.

In general the pronounced result findings in this research reflect the effect of short-term nutritional supplementation on the target time of estrus cycle of ewes. Almost all the reproductive parameters evaluated in the present study were found to be more prominent and promising in the case of supplemented groups of ewes as compared to non-supplemented group of ewes. Based on these findings, short-term nutritional supplement just prior to mating readily enhance the reproductive performance of ewes. Many studies support the effect of short-term nutritional treatments in the reproductive performance of ewes. Nutrition can determine whether an animal ovulates or exhibits estrus (Assan, 2022). De Santiago-Miramontes *et al.* (2009) observed that satisfactory feed intake resulted in a higher proportion of insulin production,

believed to promote the uptake of glucose and the synthesis of steroid hormones by the ovary. Elsewhere, nutritional flushing improved ovulation and fetal implantation in the uterus (Fitz-Rodríguez *et al.*, 2009).

## 6. CONCLUSION AND RECOMMENDATIONS

In conclusion, this study suggests that short-term nutritional supplementation with lupin or barley has positive effects on the reproductive performance of ewes. The supplementation was associated with earlier onset and prolonged duration of estrus, increased plasma glucose concentration, higher pregnancy, and prolificacy rates. This difference may be attributed to improvements in metabolic and reproductive hormone concentrations, follicular development, and oocyte quality. Notably, feeding lupin was found to be more effective in improving the reproductive performance of ewes compared to barley, potentially due to the quality of the diet. The improved reproductive performance observed in this study with short-term nutritional supplementation of ewes is highly promising. These findings provide valuable alternatives for enhancing the reproductive efficiency of sheep flocks through the manipulation of ewe nutrition. This effectively broadens the options for "focus feeding" and offer strategies for reducing the risks associated with hormonal treatments.

Based on the findings of the present study, the following recommendations are forwarded:

- Sheep producers should implement a short-term feeding schedule that provides high protein and energy nutrients just prior to mating to optimise reproductive efficiency.
- Incorporating lupin into the diet could be a promising strategy for improving the reproductive efficiency of sheep flocks.
- Targeted nutrition that meets the “clean, green, and ethical” standards has to be pioneered in the country in order to optimise the reproductive success of sheep production.
- Further research involving larger numbers of animals may be needed to prove the efficacy of this focused feeding technique under field conditions.

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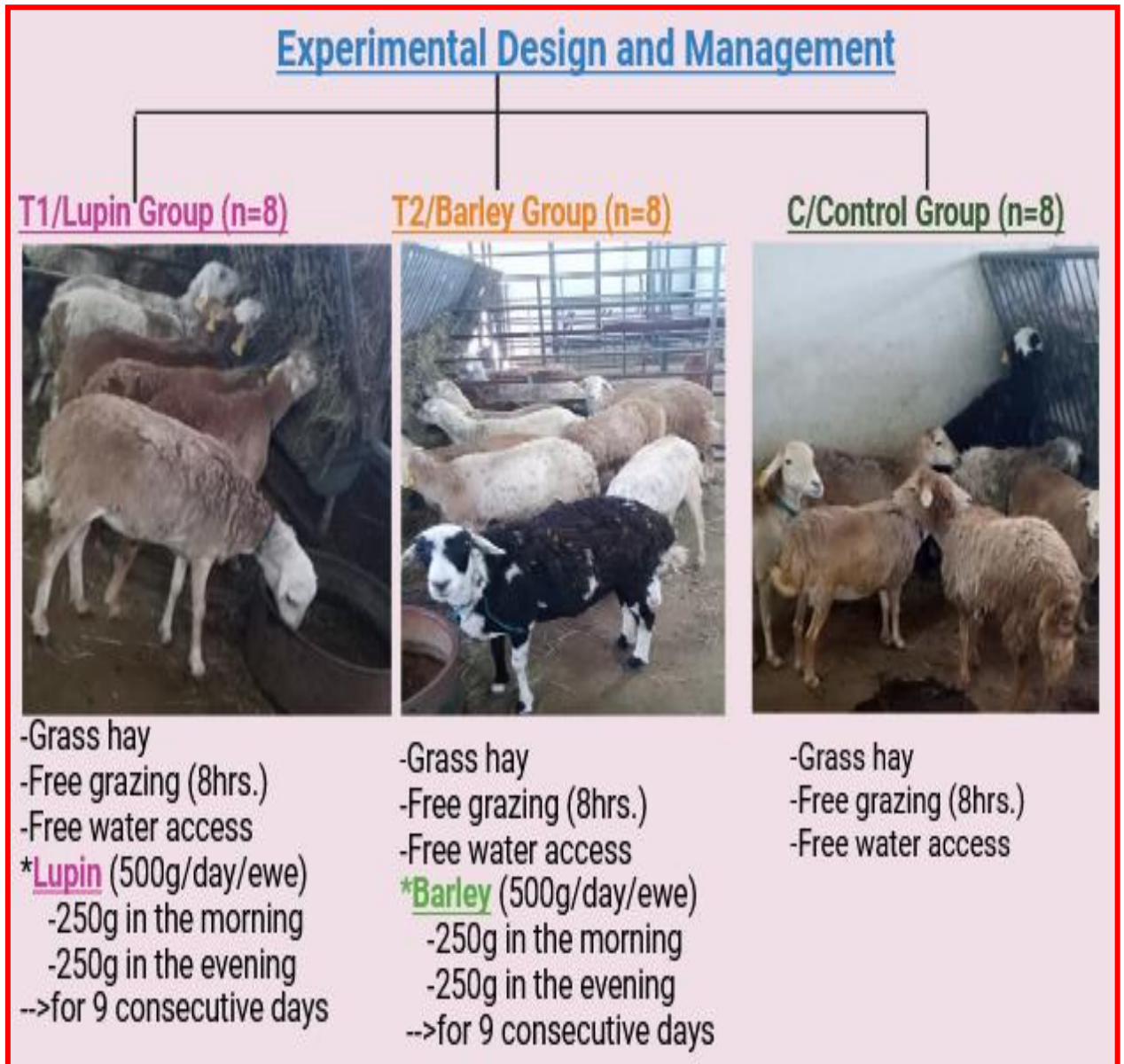
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## 8. ANNEXES

### Annex 1: Experimental Design and Animal Management



## Annex 2: Ultrasonographic Procedure

Ewes were physically restrained in standing position by the assistant

The faecal pellet was removed from the rectum

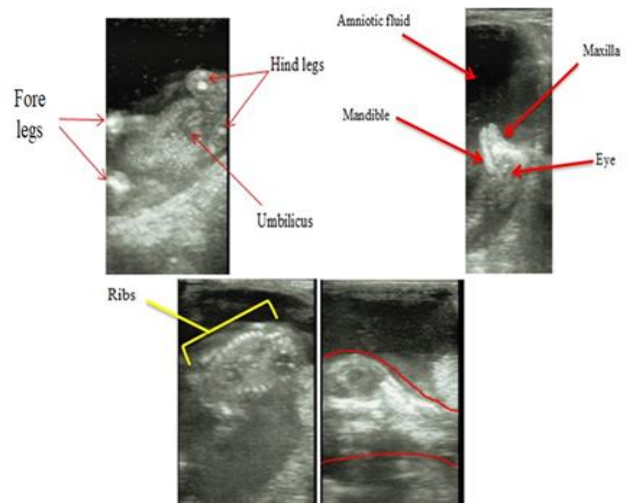
Ultrasound gel was inserted into the rectum to act as a coupling medium between the rectal wall and the transducer.

Linear array rectal ultrasound transducer with 7.5 MHz is then inserted with the lens lubricated by gel

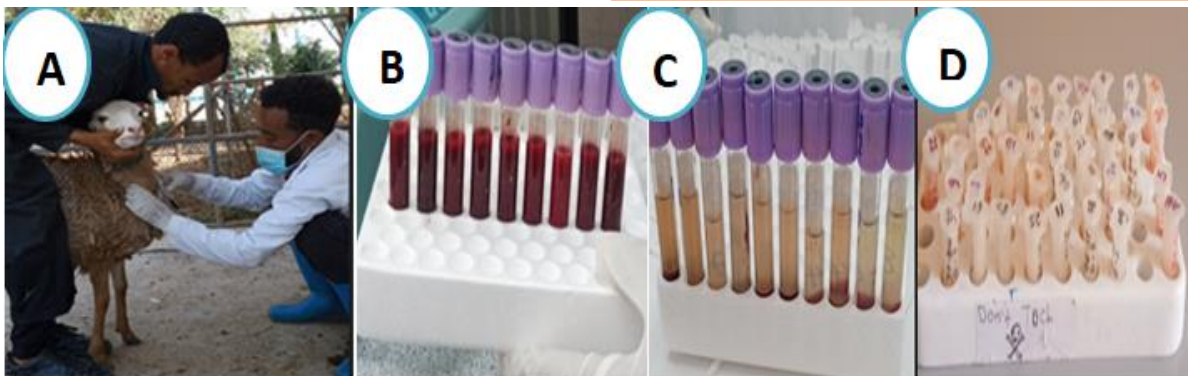
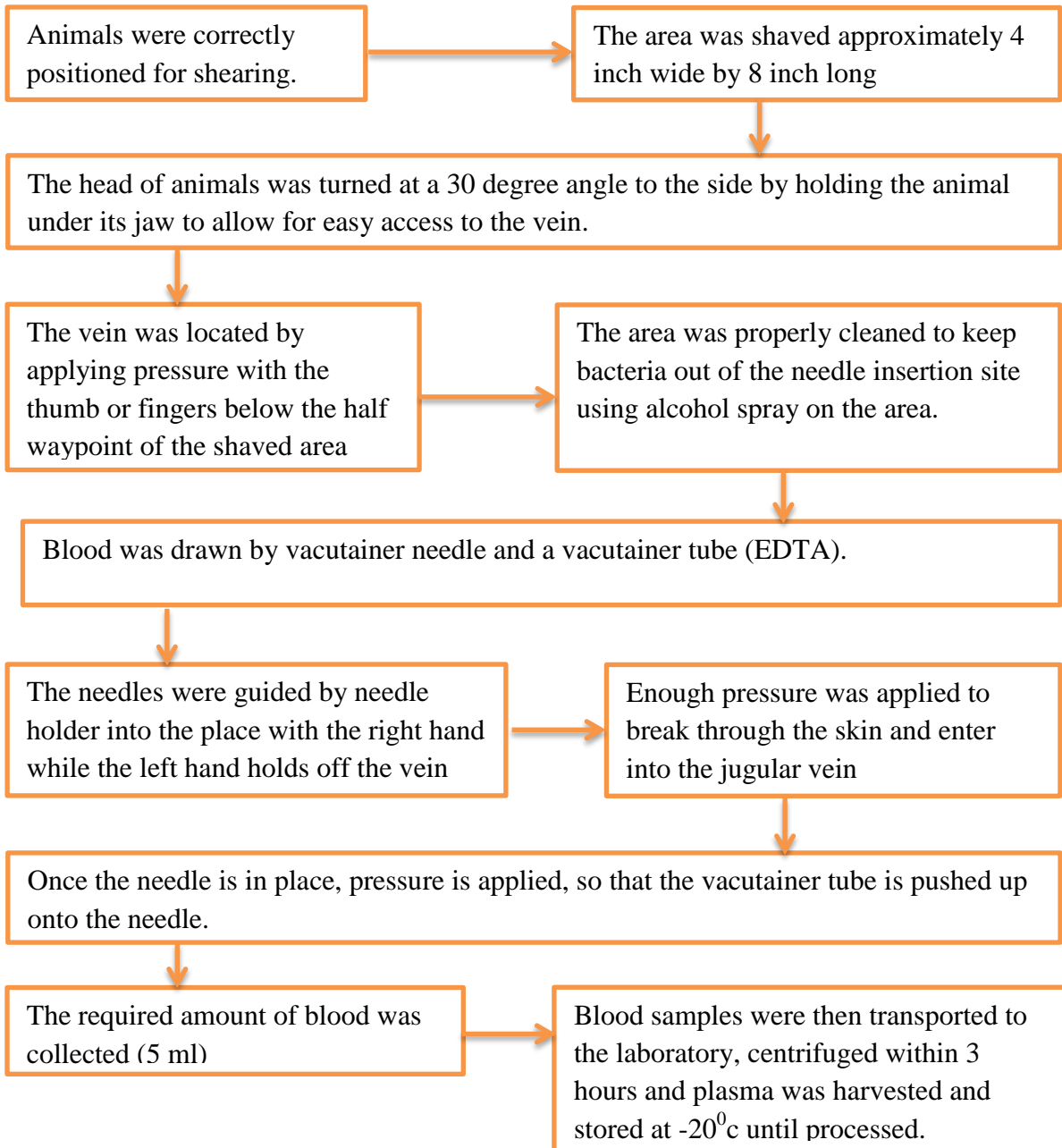
Urinary bladder was always taken as a reference point to direct the transducer towards the uterus and related target structures.

The ultrasound probe or transducer was gently moved until a clear image of desired part was seen

The desired images were frozen on the ultrasound screen, interpreted and saved



### Annex 3: Blood Sampling Procedure



#### Annex 4: Estrus Detection and Mating of Ewes

In each part of the study, behavioural observations were conducted every day from morning 12:00 to 1:00, from afternoon 7:00 to 8:00, from evening 2:00 to 1:00

Ewes were considered as in estrus when they “stand to be mounted” by vasectomised rams. Other signs such as vigorous tail-wagging, restlessness, frequent bleating and frequent adoption of urination were also considered

Finally ewes were mated naturally using Dorper breed rams

#### Estrus detection and mating

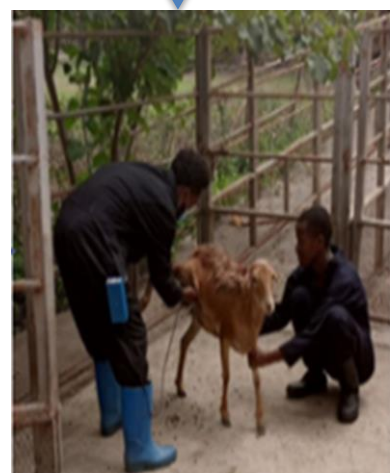
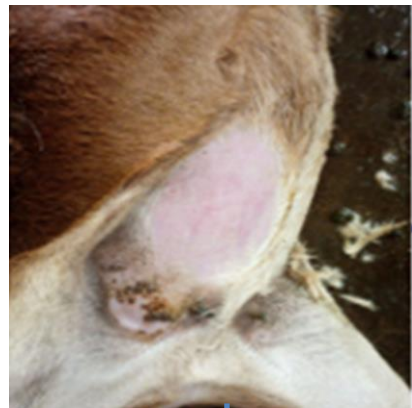
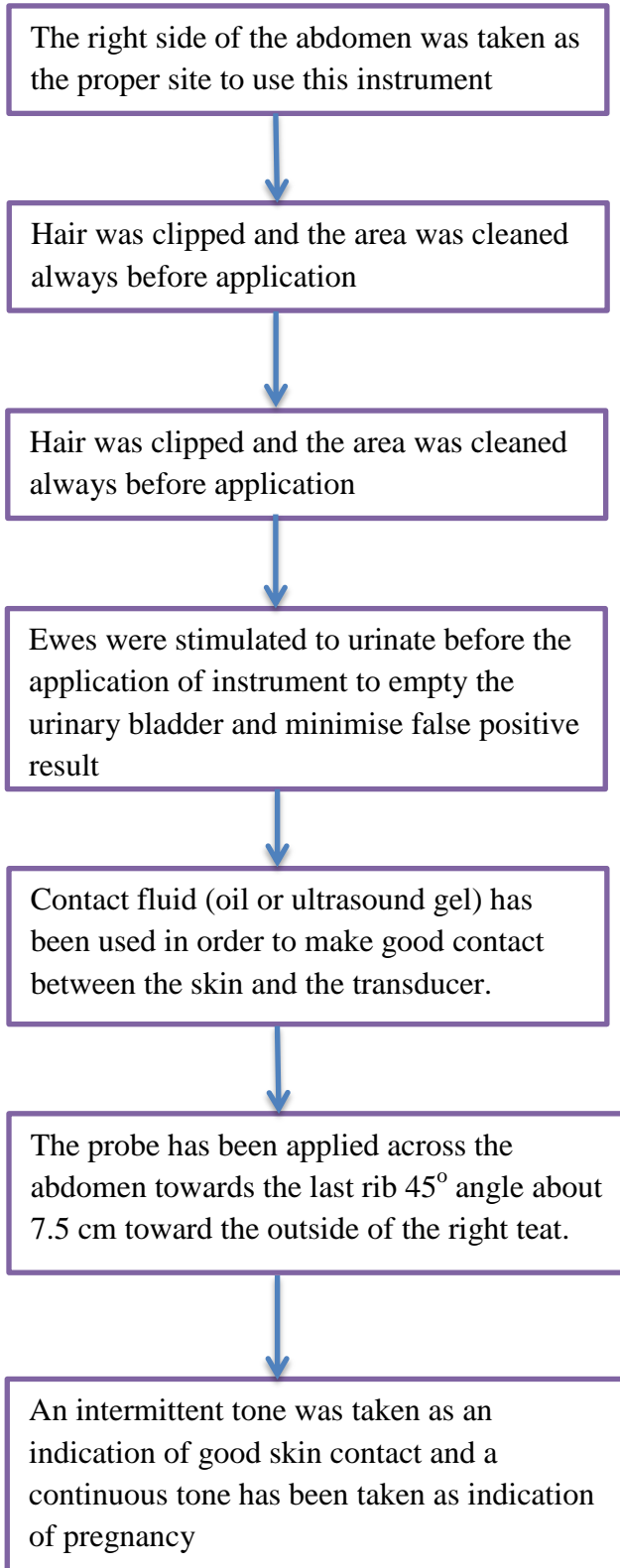


-Estrus was detected by **vasectomised** ram  
-All signs of estrus were observed & recorded



-Ewes were allowed to mate with **Dorper** breed rams  
-Mating was allowed upto the ewes desire

## Annex 5: Pregnancy Diagnosis Using Pregtone





## Annex 7: Plagiarism Certificate

# EVALUATION ON THE MODULATION EFFECT OF HIGH PROTEIN AND ENERGY DIET ON ESTRUS CHARACTERISTICS, PROLIFICACY AND PREGNANCY RATE IN MENZ BREED SHEEP

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