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**ESTRUS CHARACTERISTICS OF MENZ BREED EWES AND EFFECT OF ESTRUS
CYCLE PHASES AT INITIATION OF OVULATION SYNCHRONIZATION ON
PREGNANCY RATE.**

MVSC Thesis

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BESHOFITU, ETHIOPIA

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**A thesis submitted to Addis Ababa University College of Veterinary Medicine and
Agriculture in partial fulfillment of Masters of Science in Theriogenology**

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First, I declare that this thesis is my original work and that all sources of materials used for this thesis have been duly acknowledged. This has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University or College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for award of any academic degree, diploma, or certificate.

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ABBREVIATIONS

AI	Artificial Insemination
CL	Corpus Luteum
CSA	Central Statistical Authority
D	Day
DF	Dominant Follicle
E2	Oestradiol-17 β
ECLIA	Electrochemiluminescence Immunoassay
FSH	Follicle-Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
LH	Luteinizing Hormone
P4	Progesterone
PGF2 α	Prostaglandin
Rpm	Revolution per Minute

ABSTRACT

The study was conducted from January to April 2024 with twofold objectives: to characterize estrus signs and the time of occurrence of estrus events in natural and PGF2 α -induced estrus; and to assess the effect of estrus phase at the initiation of ovulation synchronization on plasma progesterone, plasma estradiol, and pregnancy rate in Menz breed ewes. To attain the objectives, two experiments were conducted. Briefly, in the 1st experiment, ewes (n = 12) were kept with vasectomized ram to get data from natural estrus, after which ewes were estrus induced by PGF2 α to gather induced estrus data. In the 2nd experiment, ewes (n = 12) were estrus induced by PGF2 α , and when ewes showed estrus (taken as D0 of the experiment), they were divided into two groups (n = 6 per group), balancing for age, live weight, and parity. In the 1st part of experiment two, ewes were treated with intramuscular (IM) injection of 50 μ g GnRH agonist on day 16th of estrus (at follicular phase of estrus, designed as follicular group); at day 7 of GnRH injection, ewes were IM injected with 250 μ g PGF2 α ; and at 48 hours of PGF2 α (day 9), ewes were IM injected with 50 μ g GnRH. In 2nd part of experiment two, ewes were treated in a similar protocol as in the 1st part of experiment two, but the 1st GnRH injection was started on the 8th day of the estrus (at the luteal phase of the estrus, designed as a luteal group). In both protocols, ewes were kept with a vasectomized ram, and they were bred by Dorper rams standing estrus. The behavioral and physical signs of estrus were similar to those of natural and induced estrus. Estrus events were not affected ($P > 0.05$) by estrus source, although mean estrus duration was slightly longer in natural estrus (34.1 ± 2.56 hrs) than induced estrus (32.85 ± 3.540 hrs). The mean duration of natural estrus, interval from the onset of estrus to standing estrus, duration of standing estrus, and interval from standing estrus to estrus end were 34.1 ± 2.56 hrs, 17.14 ± 3.52 hrs, 4.3 ± 0.5357 hrs, and 12.07 ± 1.08 hrs, respectively. The mean estrus cycle length was 15.46 ± 1.22 days. The luteolysis rate to PGF2 α was 100% in the follicular group within 24hrs of PGF2 α injection (P4 changed from ≥ 1 ng/ml to < 1 ng/ml within 24hrs), however, in the luteal group, all ewes underwent spontaneous luteolysis before PGF2 α (P4 < 1 ng/ml on D7). All ewes in the luteal group did not ovulate to D0 GnRH (P4 remains ≥ 1 ng/ml) while in the follicular group all ewes were spontaneously ovulated before D0 GnRH (P4 < 0.5 ng/ml at D0 GnRH). In the luteal group, the ovulation rate to D9 GnRH was 83.33% (P4 changed from > 0.5 ng/ml to $P < 0.5$ ng/m), while in the follicular group, all ewes were ovulated (100%) to D9 GnRH. The mean \pm SE P4 concentrations were 0.448 ± 0.325 ng/ml at D0 and gradually increased to

1.348±0.162 ng/ml on D7 in the follicular group; however, the mean ±SE P4 concentrations were 2.464 ± 0.355 ng/ml at D0 and gradually decreased to 0.252±0.103 ng/ml on D7 in the luteal group. Mean plasma E2 concentration was significantly lower (P<0.05) on D7 (15.48±0.87pg/ml in luteal vs. 23.84±2.641pg/ml in follicular) and D8 (16.685±0.95pg/ml luteal vs. 28.104±2.93pg/ml in follicular, P<0.05) luteal group than follicular group. The pregnancy rate was significantly lower (50%) in the follicular group than in the luteal (83.33%) group (P<0.05). Based on this preliminary study, it could be concluded that the estrus phase at the intuition of ovulation synchronization affects the pregnancy rate in Menz ewes. Moreover, when the protocol was started on the 16th day of estrus (follicular group), ewes were already ovulated, which led to a plasma average P4 level below mid-luteal stage (1.348±0.162 ng/ml) 7 days later (at PGF2 α), low pre-ovulatory P4, and a low pregnancy rate. When protocol was started on the 8th day of estrus (the luteal group), ewes underwent spontaneous luteolysis 7 days later (at PGF2 α), although the protocol resulted in higher ovulation to D9 GnRH, higher pre-ovulatory P4, and a higher pregnancy rate. To get the best starting day for ovulation synchronization, we recommend a detailed study on the length of estrus stages (pro-estrus, estrus, met-estrus, and di-estrus) and the pattern of corresponding hormonal dynamics per stage (P4, E2, FSH, LH pulse/surge) throughout the estrus cycle of Menze ewes. To overcome the spontaneous ovulation and spontaneous luteolysis encountered in this experiment we recommend a study that asses preferable days to start ovulation synchronization that would provide better pregnancy rate in Menz breed ewes.

Key words/phrase: *Estradiol, Estrus cycle, Menz ewes, Ovulation, Progesterone, Prostaglandin*

1. INTRODUCTION

Ethiopia is home to a diverse range of sheep populations, including at least 9 breeds and 14 traditional sheep populations (Gizaw *et al.*, 2007; Getachew, 2008; Takele, 2016). It is estimated that Ethiopia has 42.9 million sheep (CSA, 2021) which are largely contributed to the economy (Leta and Frehiwot, 2014). The Menz sheep breed is well-suited to the cold climate of the cool highlands, tolerant to drought, variable feed availability, endo-parasite infection, and produces meat, wool, skin, and manure (Tibbo, 2006; Getachew, 2008).

The estrous cycle in sheep ranges from 13-19 days (Rosa and Bryant, 2003), with a higher percentage of ewes having a cycle of around 17 days, with some having shorter or longer cycles (Nakafeero, 2018). The follicular and luteal phases constitute four stages of the estrous cycle. The pro-estrus and estrus stages are frequently referred to as the follicular phases (Talukder *et al.*, 2018). The luteal phases are frequently used to refer to metoestrus and dioestrus (Barrett, 2002).

In sheep, as in other ruminant species, the use of assisted reproductive technologies like artificial Insemination (AI) and multiple ovulation and embryo transfer (MOET) constitute the most effective and efficient strategies to improve the number of offspring from valuable animals and, therefore, to increase genetic gain. The success of both reproductive technologies is largely dependent on the interaction between the ovarian status and the hormonal treatments needed for allowing their application (progesterone for inducing and synchronizing estrus occurrence and gonadotrophins for inducing follicular growth and ovulation) (Gonzalez-Bulnes *et al.*, 2004; Menchaca *et al.*, 2018; Daly *et al.*, 2020).

Fixed-time AI can be achieved by understanding the time from estrus onset to ovulation in natural estrus and hormone-induced estrus. The synchronization technique for estrus should not only establish synchrony but also ensure adequate fertility levels in the synchronized cycle (Gidena, 2017). Anestrus ewes have greater control opportunities during the luteal phase, which can be extended or shortened by supplying progesterone or prematurely regressing corpora lutea (Yavuzer *et al.*, 2014). Successful techniques require tight synchrony and acceptable fertility for artificial insemination or natural mating can be achieved by combined use of gonadotropin co-treatments

with PGF2 α in cycling (Gidena, 2017). Estrus synchronization can adjust the delivery time of ewes, shorten the lambing cycle, achieve two to three deliveries in a 2 years period (Blache and Martin, 2005; Wei *et al.*, 2016; Bielhl *et al.*, 2019) there by reducing the economic cost of breeding, increasing the adjust of lambing and obtaining more economic benefits (Rosasco *et al.*, 2019).

In temperate regions where ewes can be seasonally anestrus and the induction of ovarian activity during seasonal anestrus is best achieved by using progesterone priming. In Ethiopia the day length is not vary by season and ewes can be bred at any time if the management is adequate. The use of progesterone and estrogen in animal production raises consumers concern regarding negative health and environmental effect (Martin *et al.*, 2004; Dardente *et al.*, 2016). Also the intravaginal progesterone that is used to synchronize estrus in ewes is expensive and unavailable to use under extensive sheep production. So these conditions motivated the development of ovulation synchronization methods without the use of progesterone. Several protocols that combine gonadotrophin releasing hormone (GnRH) and prostaglandin F2 α (PGF2 α) have been used with reasonable success in sheep to synchronize estrus and ovulation without the use of progestogens during breeding season (Deligiannis *et al.*, 2005).

In Ethiopia, Rekik *et al.* (2016) compared estrus and pregnancy rate in Menz ewe using progesterone priming, GnRH-PGF2 α -GnRH and double injections of PGF2 α at 11 days apart protocols with a natural mating of ewes in estrus. In this work based on plasma progesterone all ewes except one were ovulated to all the three protocols however pregnancy rate at induced estrus was varied by protocol and lower for progesterone primed ewes than those that received PGF2 α and GnRH-PGF2 α -GnRH. In other work on Menz breed ewes, Besufkad *et al.* (2020) applied fixed time artificial insemination using freshly collected semen in Menz ewes, in this work the authors compared conception rate to progesterone priming and PGF2 α protocols. Conception rate to double PGF2 α at 11 days interval yielded higher conception rate in this experiment.

The study of Rekik *et al* (2016) and Besufkad *et al.* (2020) indicated that ovulation synchronization and fixed time artificial could be a tool for intensive lamb production to obtain more than one lambing per ewe per year in Menz sheep, however, in the work of Rekik *et al* (2016) despite large estrus response and normal pattern of luteal function (P4 based) lambing rate was low (slightly

over 50%) in progesterone primed ewes. Similarly in the work of Besufkad *et al.* (2020) there was variation in conception rate among the protocols. These authors did insemination or natural mating on detected estrus, however, ovulation synchronization protocols that allow to use fixed time insemination yet be searched for Menz ewes

Many factors can be mentioned for a low conception rate to estrus synchronization including the type of protocol used for synchronization, presence of stress, nutritional status, environmental aspect and ewe's breed (Boscos *et al.*, 2002; Amarnntidis *et al.*, 2006). Fertility is related positively to concentration of progesterone during exogenous treatment (Johnson *et al.*, 1996). In addition to factors mentioned herein above the days in estrus (estrus phase) at which synchronization initiated may affect conception rate especially in protocols that utilize GnRH since variation in ewes estrus cycle and variation in number of follicular waves may be challenging to GnRH induced ovulation, The GnRH applied in synchronization protocol causes ovulation only if there is dominant follicle.

After induced ovulation to GnRH the estradiol concentration is dramatically decreased which results in FSH surge causing recruitment of a new wave follicular growth 1 to 2 days latter (Adams *et al.*, 1992) and with this new wave of follicular growth follicular development is more effectively synchronized. In cattle as number of follicular wave affects the effect of GnRH and induced ovulating (Viana *et al.*, 2000; Martinez *et al.*, 2000). To our knowledge, there are only a few studies that have assessed the effect of estrus phase on GnRH ovulation induction and the related pregnancy rate in sheep in general and Menz ewes in particular.

Based on the facts mentioned herein above we hypothesized the following;

- 1 Estrus characteristics and time events of estrus in Menz sheep may vary between natural estrus and PGF2 α induced estrus. Understanding the time of estrus events specially the ovulation time in relation to standing estrus, estrus onset and end of estrus have paramount importance in timing artificial insemination.
2. The phase of estrus cycle at which the initial GnRH is given in GnRH- PGF2 α –GnRH ovulation synchronization protocol may affect ovulation rate and pregnancy rate in Menz ewes. In cattle elevated P4 concentrations suppress LH release and are linked with low ovulatory response to the

initial GnRH treatment in ovulation synchronization protocol. We want to prove or disprove this in Menz breed ewes.

The objectives are:

- To characterize details of Menz breed ewe's natural estrus signs.
- To compare events of estrus and their time of occurrence in natural estrus with PGF2 α induced estrus
- To evaluate effect of estrus phase at the initiation of ovulation synchronization on plasma progesterone and estradiol during treatment course in Menz breed ewes
- To evaluate effect of estrus phase at the initiation of ovulation synchronization on pregnancy rate in Menz breed ewes
- To evaluate luteolysis rate to PGF2 α .

2. LITERATURE REVIEW

Compared to large ruminants, sheep have shorter production cycles, higher growth rates, and greater environmental adaptation, which gives them an advantage in smallholder agriculture (Tibbo, 2006). Uncontrolled breeding and mating lead to random lambing that occurs all year. As a result of lambing during an unfavorable season, there has been a significant rate of lamb mortality. Furthermore, there were fewer lambs available for export and domestic consumption of a similar weight and age due to uncontrolled breeding (Aderajew, 2015). The country's needs for mutton sheep and prolific ewes differ greatly from the production and reproductive capacity of sheep.

It is necessary to use sheep-assisted reproductive technology to achieve a balance between output and demand. Estrus synchronization is a technology utilized globally to enhance reproductive efficiency in sheep (Vilarino *et al.*, 2017; Nakafeero, 2018). Sheep fertility decreases with estrus synchronization and artificial insemination due to inadequate AI timing in relation to ovulation and method, compared to natural estrus and natural service breeding (Fair *et al.*, 2005; Nakafeero, 2018). Synchronization of ovulation involves manipulating an animal's reproductive cycle using hormones or their analogs to induce ovulation at a precise point in time (Gidena, 2017).

Prostaglandin-based estrous synchronization initiates different follicular phases of the estrous cycle by ending the CL. The method is limited to cyclic ewes, is not suitable for inducing estrus, and is most effective during breeding season (Whitley and Jackson, 2004; Maqhashu, 2019). Understanding reproductive physiology is crucial for the successful implementation of manipulative reproductive technologies in animals (Talukder *et al.*, 2018)

2.1 Phases and Stages of the Estrous Cycles in Ewes

The hypothalamic anterior pituitary-ovarian axis controls the events of the estrous cycle, as the downstream tissue of the hypothalamus-pituitary-ovary axis (Bazer, 2020). The hypothalamus, which produces GnRH; the pituitary gland, which secretes FSH, luteinizing hormone (LH), and oxytocin; the ovarian antral follicles, which secrete estrogens and inhibin; the CL, which secretes

progesterone and oxytocin; and the uterine endometrium, which produces $\text{PGF2}\alpha$, are all involved in the estrous cycle (Bartlewski *et al.*, 2011).

Antral follicles that develop continuously until they ovulate or become atretic are indicative of patterns of ovarian follicular development in sheep. Specifically, FSH impacts the quantity of developing follicles, stimulates the ultimate maturation of follicles, and boosts follicle growth and development (Shabankareh, 2009).

The ovary plays a crucial part in the sheep's estrous cycle (Bazer, 2020). There are two phases (follicular and luteal) and four stages (pro-estrus, estrus, metestrus, and diestrus) that make up the estrus cycle in ewe. The follicular phases, which include the proestrus and estrus stages, are frequently used. The luteal phases, which include metestrus and diestrus, are frequently used (Barrett, 2002).

2.1.1 Phases of the Estrus Cycle in Ewes

Follicular phase

The follicular phase begins around the time of luteolysis, when progesterone concentration decreases and lasts for 2-3 days (Gonzalez-Bulnes *et al.*, 2005). The follicular phase involves increased estrogen levels and decreased progesterone, with the ovulatory follicles growing and producing ova. Low progesterone levels in the blood indicate the follicular phase, potentially pro-estrus or estrus (Talukder *et al.*, 2018). The peak in serum estradiol concentrations occurs at the end of the growth phase of the largest follicle of the wave, approximately on day 16 of the cycle (Talafta and Ababneh, 2011).

The size of the dominant follicle does not change much before estrus but increases significantly on the day of estrus (Nakafero, 2018). Large follicle size and number significantly affect fertility in sheep breeds, with large follicles producing more estradiol and progesterone, while small follicles may alter ovulation rates. (Letelier *et al.*, 2011; Nakafero, 2018). A further necessary hormone that encourages oocyte maturation and ovulation is LH (Wang *et al.*, 2022).

Ultrasonography studies ovarian follicles' wave-like growth pattern, with three to four waves occurring during the estrous cycle. Wave 1 emerges around ovulation day, with other waves emerging every 4–7 days, depending on the number of waves (Seekallu *et al.*, 2010; Nakafeero, 2018). The dominant follicle will secrete inhibin and suppress smaller follicle growth, leading to regression or atresia (Cueto *et al.*, 2006). However, studies in sheep show no inhibitory effect, with new follicles emerging and ovulating in large follicles.

Luteal phase

The luteal phase, initiated after ovulation, involves LH activating granulosa cells of ruptured follicles, transforming them from estrogen-producing to progesterone-producing cells, and forming the CL. The size of the CL increases during the early to mid-luteal phase, resulting in increased progesterone concentration and inhibiting LH secretion via negative feedback to the hypothalamus. The study found that progesterone secretion gradually decreases at 11-13 days of estrus, while structural luteolysis occurs at 14 days (Nakafeero, 2018).

The luteal phase of the estrous cycle can be manipulated by either prolonging it with P4 or shortening it with PGF2 α (Abecia *et al.*, 2012; Nakafeero, 2018). Progesterone, when established during pregnancy, helps maintain pregnancy by inhibiting further estrous cycles, increasing uterine gland secretion, and reducing uterine motility for successful implantation (Nakafeero, 2018). If viable embryos are absent on day 13 of the estrous cycle, oxytocin pulses activate prostaglandin F2 α secretion from the endometrium, leading to luteal tissue regression and reduced progesterone secretion (Talafha and Ababneh, 2011).

2.1.2 Stages of the Estrous Cycles in Ewes

Proestrus

The ovarian follicles start releasing a large amount of E2 on Days 15 and 16 of the pro-estrus, when CL is totally regressed. The next estrous cycle starts when the estrus first appears, and a dominant follicle or follicles are selected for ovulation (Bazer, 2020). The final maturation and development of the ovulatory follicle occur during the pro-oestrus and estrus stages after luteolysis (Barrett, 2002). When the CL undergoes luteolysis, it returns to the corpus albicans, a dormant tissue that no longer secretes progesterone and is incapable of producing viable cells (Bazer, 2020).

Estrus

The start of sexual receptivity for mating and the start of the estrous cycle are both considered to occur on Day 0 of the estrus in ewes. As a result of an estrogen-induced surge of LH and FSH from the anterior pituitary at the beginning of the estrus, spontaneous ovulation takes place about 30 hours after the initiation of the estrus and lasts for about 30 hours (Bazer, 2020).

Metestrus

Following estrus, which lasts for Days 1-4 of the estrous cycle, is metestrus, which is distinguished by the luteinization of theca and granulosa cells of the ovarian follicle under the influence of LH to first form the corpus hemorrhagicum (CH) and later the CL that secretes progesterone (Bazer, 2020). For initial CL growth and cellular differentiation, LH support is essential. The diameter of the ovine CL reaches 6-8 mm between 3 and 4 days after ovulation (Bartlewski *et al.*, 2011).

Diestrus.

In the stage of diestrus, the CL reaches its greatest size and progesterone release around days 4–14 of the estrous cycle (Bazer, 2020). About 6 days later, the ovine CL achieves its maximum diameter of 11–14 mm (Bartlewski *et al.*, 2011). Between days 4 and 12 of the cycle, the size of large luteal cells increases, but their number remains mostly stable until the start of luteolysis (Bartlewski *et al.*, 2011). Between days 12 and 15 after ovulation, each sheep has sudden luteal shrinkage over the following two to three days (Bartlewski *et al.*, 2011).

2.2 Characteristics of Estrus in Ewe

Both rams and ewes must exhibit acceptable levels of sexual contact for successful mating (receptivity in females and desire in males) (Ekiz *et al.*, 2013). Estrous is traditionally detected by rams because it is subtle and difficult to detect otherwise (de Freitas *et al.*, 2018), but breeders may find the time required for observation to be labor-intensive. A doe, but not a ewe, may occasionally display homosexual conduct. The majority of the time, ewes exhibit aggressive male-seeking behavior and stick close to the male. However, the ewe's estrus is challenging to identify when the male is not present (Jainudeen *et al.*, 2000).

The most significant and dependable indication of a female going into estrus is when the female exhibits active search behavior with regard to the male and immobility during mating (de Freitas *et al.*, 2018). The presence of a male is required because ewes do not readily exhibit indications of estrus, such as a swelling vulva or mucosal discharge via the vagina (Barrett, 2002).

Oedema and redness caused by increased blood flow in the vulvar tissues, a mucous discharge from the cervix, vagina, and uterus glands, additional thickening of the epithelial layers of the vagina and endometrium, softness of the cervical stroma, and other symptoms are all indications of estrus in ewes. Estradiol levels rise in the blood, which triggers the appearance of signs of estrus (Jainudeen *et al.*, 2000; Barrett, 2002; Talukder *et al.*, 2015).

2.3 Estrus and Ovulation Synchronization

Similar to other domestic species, sheep's reproductive management is commonly based on the pharmacological induction and synchronization of estrus and ovulation, whether during reproductive or non-reproductive seasons, for either natural mating or artificial insemination (Abecia *et al.*, 2012). Synchronization of estrus during breeding season reduces breeding time, shortens lambing period, and ensures uniform weight birth of lambs (Ali, 2007; Nakafeero, 2018). A key strategy for increasing the percentage of lambs born, managing lamb crops, and optimizing ewes' returns on investment is controlled breeding through estrus synchronization. There are multiple approaches that have been used to successfully synchronize sheep's estrous cycles (Aderajew, 2015). According to Gizaw *et al.* (2016), Ethiopian sheep breeds have estrus responses to hormonal synchronization that range from 65 to 100%.

Estrus synchronization is a method utilized to manage the estrous cycle (Farrag, 2019) and requires effective management, regardless of the protocol used (Gidena, 2017). Synchronization is achieved through hormonal treatments that mimic the corpus luteum's activity or eliminate it, promoting a follicular phase through ovulation (Abecia *et al.*, 2011), and it is a method used by ewes to adjust their ovarian cycle to release an oocyte at a specific time for AI or natural mating. Optimal fertility rates are influenced by a balance of endocrine responses (Maqhashu, 2019). Synchronization and ovulation are designed to maintain hormonal balance for optimal results post-fertilization (Lehloenya *et al.*, 2005; Maqhashu, 2019). Poor synchronization of ovulation occurs when

dominant follicles are at different stages of development during insert withdrawal due to a failure to achieve follicular wave development synchrony (Nakafeero, 2018).

A successful estrus synchronization programme offers numerous benefits, utilising ARTs for genetic improvement in livestock (Amiridis and Cseh, 2012; Gidena, 2017). Estrus synchronization allows for the use of AI, potentially enhancing genetic improvement and increasing production (Faigl *et al.*, 2012). Ovulation and reproduction can be controlled by exogenous hormonal treatment to improve breeding programmes and increase the number of pregnant females (Dias *et al.*, 2018). Successful techniques require tight synchrony and acceptable fertility for artificial insemination or natural mating, often achieved through gonadotropin co-treatments, which form the basis for successful AI and embryo transfer programmes (Gidena, 2017).

2.3.1 GnRH-PGF2 α

The hypothalamic hormone, GnRH, also known as Cystorelin®, Fentanyl®, Factrel®, and Ova Cyst®, is a naturally occurring hormone that activates the release of other hormones, including FSH and LH (Gidena, 2017). Gonadotropin-releasing hormone, FSH, and equine chorionic gonadotropin, along with the ram effect, are combined with P4 or PGF2 α to stimulate follicular development and synchronize estrus and ovulation (Delgadillo *et al.*, 2009; Nakafeero, 2018). GnRH is a hormone that impacts follicle development in the ovary and causes ovulation.

In Ethiopian native sheep breeds, the effectiveness of GnRH-PGF2 α treatment is effective in synchronizing estrus with acceptable conception rates. In the Atsbi breed, Afar breed, Humera breed, and Dangla breeds of native Ethiopian sheep, the estrus response was 100%, 66.6%, 100%, and 100%, respectively, and the conception rate was similar to that of the heat response. Regarding the hormone's effect, all breeds, with the exception of the Humera, responded fully to the initial hormone injection when given GnRH at day 0 and PGF2 α at day 7 of the experiment (Gebrekidan *et al.*, 2014). The four systems for synchronization of estrus with GnRH-PGF2 α combinations are Ovsynch, Cosynch, Select Synch, and Hybrid Synch (Islam, 2011).

Select Synch:

This combination represents the simplest GnRH-based system. A common name for the GnRH-PGF2 α system is “Select Synch” (Islam, 2011). an injection of GnRH on day 1, injecting females not artificially inseminated with prostaglandin on day 7, and then estrous detection and breeding following day 8 (Gidena, 2017).

Ovsynch:

The Ovsynch program involves GnRH injections on day 1 used to stimulate follicle growth in cyclic females and induce ovulation; PGF2 α injections on day 7 induce luteolysis of CL and decline progesterone concentration; GnRH injections on day 9 encourage ovulation of dominant follicles that have been pre-programmed by the first GnRH treatment; and timed insemination on day 10. The program offers advantages such as close-fitting synchronization of estrus, high response rates from females, and encouraging estrus in non-cycling females (Bekahegn, 2019).

CO-Synch:

The CO-Synch program is comprised of an injection of GnRH on day 1, an injection of PGF2 α on day 8, and then a second injection of GnRH with breeding on day 10 (Islam, 2011).

Hybrid-Synch:

The Hybrid-Synch program is a combination of the Select Synch and CO-Synch protocols (Gidena, 2017). The Hybrid-Synch program is implemented with an injection of GnRH on day 1, an injection of prostaglandin on day 8, and then estrous detection and breeding from day 8 to 11 (Islam, 2011).

2.3.2 PGF2 α

Prostaglandin administration is a preferred method for inducing estrous in ewes due to its rapid lungs-metabolized conversion into 15-keto-prostaglandin F2 α and 13, 14-dihydro-15-keto prostaglandin F2 α (Maqhashu, 2019). The ovary releases oxytocin, activating the production of PGF2 α (Aderajew, 2015). Prostaglandin, released from the uterus, regulates reproductive cycles in domestic species by controlling luteal activity in non-pregnant animals and initiating delivery in pregnant animals (Ricciotti and FitzGerald, 2011). Although the drop in plasma P4 concentrations

is more pronounced after luteolysis, PGF2 α must be administered in the presence of a CL for it to be effective (Abecia *et al.*, 2011).

PGF2 α can be administered to tropical sheep breeds year-round, causing regression and rapid follicular growth, estrous, and ovulation after two injections given 7-14 days apart (Contreras-Solis *et al.*, 2009; Fierro *et al.*, 2016). PGF2 α analogues exhibit up to 90% efficacy in estrus manifestation in sheep after 48 hours of estrus synchronization (Alavez *et al.*, 2014). The estrous response and fertility after prostaglandin treatment are influenced by factors such as dose level, CL responsiveness, estrous synchronization stage, season, and co-treatments with gonadotrophins (Maqhashu, 2019).

3. MATERIALS AND METHODS

3.1 Study Area

The study was carried out on the premises of the College of Veterinary Medicine and Agriculture of Addis Ababa University, which is located in Bishoftu. Bishoftu Town is located 47 km from the capital of Addis Ababa in the East Shewa Zone of the Oromia National Regional State. The city is located at 1870 metres above sea level. It has an annual rainfall of 866 mm, of which 84% is in the long rainy season (June to September) and the remaining in the short rainy season (from March to May). The dry season extends from October to February. The mean annual maximum and minimum temperatures of the area are 26°C and 14°C, respectively, with a relatively high mean humidity of 61.3% (Girmay *et al.*, 2022).

3.2 Study Animals

For this experiment, 12 Menz breed ewes and 3 rams were used. One vasectomized Menz breed ram (annex 5) was used to detect estrus, while two dorper rams were used for cross-breeding. Before the start of the experiment, the sheep were ear-tagged and dewormed every month with anthelmintic (Triclabendazole Bolus 250 mg/25 b.w.t. Fasinash®sheep, Ashish Life Science Pvt. Ltd., India) and Ivermectin Injection 1%, 1 ml. per 50 kg. Bodyweight, China. The ewes were dry and non-lactating, and they were examined for any reproductive problems (by vaginal speculum and transrectal ultrasound, Aloka, Japan) and for external physical injuries. The mean age of ewes was 2.16 ± 0.27 years (from farmer and estimated using dentation (annex 1)), the mean parity of ewes was 1.75 ± 0.304 , and the mean body weight was 24.25 ± 0.72 kg.

3.3. Housing and Feeding System

All experimental ewes were housed in the animal house at CVMA in groups. The sheep were allowed to graze for at least 10 hours during the day and were provided with grass hay and fresh water ad libitum when kept in the house (fig. annex 6, plate 1).



Figure 1: Housing and feeding system of the experimental sheep: **A)** Grazing during the day; **B)** Feeding (hay) during the night.

3.4. Experimental Design

This work has two parts. In the first part, the ewes ($n = 12$) were kept with the vasectomized ram during the day and night. In addition to the detection of estrus expression by vasectomized rams, the ewes were visually observed by one researcher every 3 hrs for 30 minutes (6 a.m., 9 a.m., 12 a.m., 3 p.m., 6 p.m., 9 p.m., 12 p.m., and 3 a.m. in local time) for the expression of natural estrus for two consecutive estrous cycles.

Time to onset of estrus, duration of standing estrus (measured by hours between the first and last standing estrus), and estrus cycle length (measured by the duration between the onsets of two consecutive estrous periods), as well as estrus duration, were characterized.

The vasectomized ram helped in the estrus detection in such a way that the ewe stands still when approached by a ram, head and ear position (swinging head back and looking at the ram), remain standing when nudged by the ram, walking away, or acceptance of service were indicators of the ewe being in estrus. Ewe were watched for signs of high estrus intensity, during which the ewe initiated the mating by nuzzling the ram's forequarters, pushing her head into the ram's flank, and her nose directed to the scrotal area. The time of each of these signs from the start of estrus were recorded (fig. annex 6, plate 2). The rams were painted daily on the brisket area with oil based pigmented dye. The ewes were watched on the rear quarters for the dye mark made by mating of the ram, and this helped to establish cyclical episodes of estrus in ewes. Observations were made in a way that would not interfere with the normal behavior of the animals.

3.5 Collection of Blood Samples

Blood (4 ml) was collected aseptically from the jugular vein into vacuum blood tubes with EDTA (No. 8, Xiyuan Road 3, Westlake District, and Hangzhou, Zhejiang, China) from all ewes in both groups. The area was shaved, cleaned, and disinfected with a soaked cotton swab before blood collection. Blood samples were collected every 48h from day zero (at GnRH injection) until day four, and then at day seven (at PGF2 α injection) for P4 analysis. From day seven until 11, blood was collected every 24h for P4 and E2 analysis. After collection, the blood samples were labelled and brought to the laboratory in a thermocol box containing ice packs. Plasma was separated by centrifuging at 3000 rpm for 10 min. Then, the plasma sample was transferred into plastic 2.0-mL micro-centrifuge tubes using sterilized plastic disposable pipettes and then stored at -20 °C until assayed for P4 and E2 analysis (fig. annex 6, plate 3). Plasma P4 and E2 analysis was done at EPHI using electrochemiluminescence immunoassay (ECLIA) (Cobas 6000, Roche) (annex 2).

The measurement of ovarian activity was performed based on the analysis of plasma levels of progesterone, effectively declaring ewe in sexual activity at a concentration ≤ 1 ng/ml (Boscos *et al.*, 2003; Laghrour *et al.*, 2020).

3.6 Pregnancy Diagnoses

Pregnancy rates were checked after days 35. On ultrasound, the presence of a fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of conception. The examinations were performed using transrectal ultrasonography (Aloka, SSD-500, Japan) equipped with a 7.5-MHz linear transducer with ewes in a standing position. Faecal pellets were removed digitally, and hydrosoluble gel was filled into a syringe and deposited into the rectum to act as a coupling medium between the rectal wall and transducer. The transducer was inserted and manipulated in the rectum by external control of the extension. The urinary bladder, cranial vagina, and cervix were viewed in the longitudinal planes while the transducer was being inserted. After the cervix and caudal uterus were viewed, the embryo appeared as an echogenic mass surrounded by anechoic fluid within the uterine lumen. Placentomes were detected as small gray C- or O-shaped structures around the edges of the fluid-filled vesicle (Talafha and Ababneh, 2011) (fig. annex 6, plate 4).



Figure 4: Pregnancy diagnosis: A) Trans-rectal pregnancy diagnosis in ewes;B) Presence of a small gray O-shaped structure (placentomes) in the ewe's uterus.

3. 7 Data Collection and Analysis

The estrus response rate was calculated as the number of ewes showing signs of estrus per total ewe. Duration of standing estrus was measured by hours between the first and last standing estrus; total estrus duration was time between the 1st observed sign and the last estrus sign; and estrus cycle length was measured by the duration between the onsets of two consecutive estrous periods. The ovulation rate is calculated by dividing the number of ewes that ovulate by the number of ewes in estrus. The induced estrus response rate after $\text{PGF2}\alpha$ treatment was measured by dividing the number of ewes in estrus by the number of ewes receiving $\text{PGF2}\alpha$. Luteolysis rate is measured by dividing the number of ewes with $\text{P4} < 1 \text{ ng/ml}$ after $\text{PGF2}\alpha$ treatment by the total number of ewes that have a P4 concentration $> 1 \text{ ng/ml}$ and have received $\text{PGF2}\alpha$ treatment. The pregnancy rate was measured by dividing the total number of ewes that were pregnant by the total number of ewes that were mated. Comparisons between treatment groups were made using a t-test in STATA software. Descriptive statistics were used to compute rates.

4. RESULTS

4.1 Estrus Characteristics

The mean estrus cycle length of Menz breed ewes was 15.46 ± 1.22 days (range 12.79 to 18.12 days) in this study. The mean estrus duration was slightly longer in the natural estrus (34.1 ± 2.56 hrs) than the induced estrus (32.85 ± 3.54 hrs), although the difference was not significant ($P > 0.05$). The estrus induction rate to PGF2 α was 63.64%, while the remaining 36.36% of ewes did not respond to a single PGF2 α treatment (Table 1).

Table 1: Comparison of estrus duration by estrus source in Menz breed ewes

Estrus duration & luteolysis rate \dagger	Estrus source			
	Natural estrus		Induced estrus	
	Mean \pm SE	Range	Mean \pm SE	Range
Duration of Estrus(hrs)	34.1 ± 2.56	28.30-39.89	32.85 ± 3.54	24.19-41.52
Onset to standing estrus (hrs.)	17.14 ± 3.52	8.52-25.75	16.28 ± 2.18	10.92-21.64
Standing estrus duration (hrs.)	4.3 ± 0.5357	3.12-5.58	2.8 ± 0.48	1.43-4.16
Standing to ends of estrus (hrs.)	12.07 ± 1.08	9.71-14.43	15.16 ± 2.15	9.63-20.69
Estrous cycle length (days)	15.46 ± 1.22	12.79-18.12	NM	NM
Estrus response rate	100% β	NA	63%	NA

\dagger = at P value 0.05 there was no statistically significant difference across the rows for all estrus events duration, β = Natural estrus & all ewes showed estrus, hrs = hours, NA = not applicable, NM = not measured.

4.1.1 Behavioral Estrus Sign in Menz Ewes

Sniffing the ram genital area and forequarters, running, frequently swinging head back and looking at the ram face, restlessness, frequent urination, pushing head into the rams flank, standing to be mounted, mounting to other ewes, and remaining close to the ram were among the behavioral signs that were observed during this study time.



Figure 5: Behavioral estrus sign. A) Sniffing scrotal area B) pushing head into the rams flank

4.1.2 Physical Estrus Sign in Menz Ewes

The physical estrus signs observed include flagging tail rapidly, rapidly moving tail, vaginal discharge, vulva swelling, and reddening, and all ewes that were in estrus had shown these physical estrus signs (Fig 6).

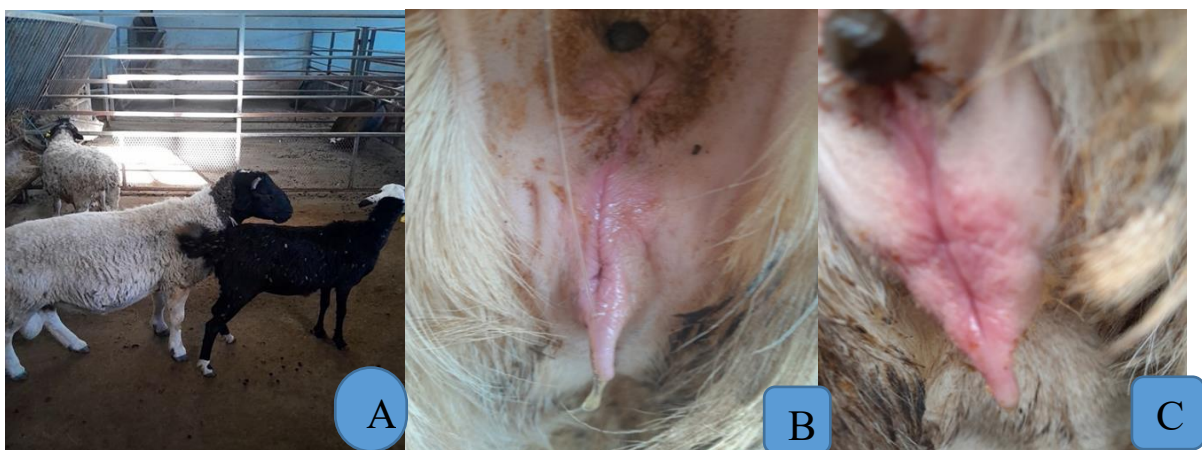


Figure 6: Physical estrus sign A) flagging tail and remaining in front of the ram B) vaginal discharge C) vulva swelling and reddening.

4.1.3 Estrus Signs by Estrus Duration

Estrus sign during the onset estrus

At the onset of the estrus, the rams detected the ewes by sniffing the anogenital area, and tried to mount several times; however, the ewes didn't remain in standing, which means the ewes were not sexually receptive. The estrus sign during the onset of estrus includes sniffing the ram's genital area and forequarters, running away when the ram tried to mount, looking at the ram's face, restlessness, and frequent urination, rapidly moving tail, and mounting other ewes. These were the signs that were recorded during the onset of estrus.

Estrus sign during standing estrus

During standing estrus, all ewes were sexually receptive. Before mounting, the ram prepared the ewes (sniffing the anogenital region, contacting the flank of the ewes, and striking out at the female with one front leg), and finally the ewe prepared herself (flagging her tail and remaining in front of the ram) for successful mating. The signs that repeatedly occurred during the standing periods include flagging tail rapidly and moving, standing to be mounted (sexually receptive), following the ram and remaining close to the ram, sniffing the genital area and forequarters frequently, vaginal discharge, vulva swelling and reddening, frequently swinging head back and looking at the ram's face, pushing head into the ram's flank, restlessness, frequent urination, and mounting to other ewes.



Figure 7: Behavior at standing estrus; **A)** The ram prepare the ewe, and ewe prepare herself **B)** Standing to be mount.

Estrus sign after standing period

Estrus signs after standing estrus to the end of estrus include the ram tried to mount several times, but the ewes were running away because they were not sexually receptive (they did not remain standing). The ewes began to separate from the ram. However, sniffing the genital area and forequarters, looking at the ram's face, the vulva swelling, and moving tails were still observed after standing estrus.

4.2 Initiation of Ovulation Synchronization and Response Rates

In part two, the ewes were given GnRH and PGF2 α at different estrus phases; the estrus response rate to PGF2 α in both the follicular and luteal phases' groups was 100%. Additionally, there was no significant difference ($P > 0.05$) in time from the PGF2 α to the onset of estrus and the duration of estrus by group. The signs of estrus behavior were also similar to those of natural estrus behavior.

4.2.1 Luteolysis Rate to PGF2 α Treatment

Complete luteolysis was defined as ewes with plasma P4 ≥ 1 ng/ml at PGF2 α (D7) and plasma P4 < 1 ng/ml after PGF2 α in subsequent days. In the luteal group, all ewes underwent 100% spontaneous luteolysis before PGF2 α (P4 < 1 ng/ml on D7). The effectiveness of PGF2 α treatment to cause luteolysis in the follicular group was 100%, and luteolysis was completed within 24 hrs of PGF2 α injection (P4 changed from ≥ 1 ng/ml to < 1 ng/ml within 24hrs).

4.2.2 Ovulation Time and Ovulation Rate to GnRH

Responses to GnRH were significantly ($P < 0.05$) influenced by the estrus phase at the initiation of ovulation synchronization on study D0. All ewes in the luteal group did not ovulate to D0 GnRH (P4 remains ≥ 1 ng/ml). In the luteal group, the proportion of ewes that were ovulation to D9 GnRH was 83.33%, from which three ewes were ovulated between 24 to 48hrs of GnRH (P4 changes from > 0.5 ng/ml to $P < 0.5$ ng/ml between 24 and 48hrs of GnRH) and two ewes were ovulated within the 1st 24hrs of GnRH. One ewe was ovulated before D9 (before 2nd GnRH). One ewe showed continuously increasing P4 concentration and was removed from ovulation data. In the follicular group, all ewes were spontaneously ovulated (P4 < 0.5 ng/ml) on D0 GnRH, and all ewes were

ovulated on D9 GnRH. Three ewes were ovulated within the 1st 24hrs of GnRH, while three ewes were ovulated between 24 and 48hrs of GnRH.

4.2.3 P4 ng/ml Concentration Results

The Mean±SE P4 concentration at D0 (start of experiment) where the ewes received 1st GnRH was 0.448 ± 0.325 ng/ml for the follicular group and 2.464 ± 0.355 ng/ml for the luteal group ewes. From D0 onward, the Mean±SE P4 gradually increased and was 1.348 ± 0.162 ng/ml on D7 (day of PGF2 α) in the follicular group; however, in the luteal group, the Mean±SE P4 gradually decreased and was 0.252 ± 0.103 ng/ml on D7 (day of PGF2 α). The Mean±SE P4 concentration was 1.348 ± 0.162 ng/ml at PGf2 α in the follicular group and was changed to 0.120 ± 0.019 ng/ml 24hrs later (D8), which indicated fast luteolysis. At D11, the Mean±SE P4 concentration was raised to 0.141 ± 0.027 ng/ml which marks the start of met-estrus. For the luteal group, Mean±SE P4 concentration was 2.568 ± 0.264 ng/ml at D4 and changed to 0.252 ± 0.103 ng/ml at PGF2 α (D7), which indicates luteolysis spontaneously occurred before day seven (Fig. 8 DF from the new wave was ovulated).

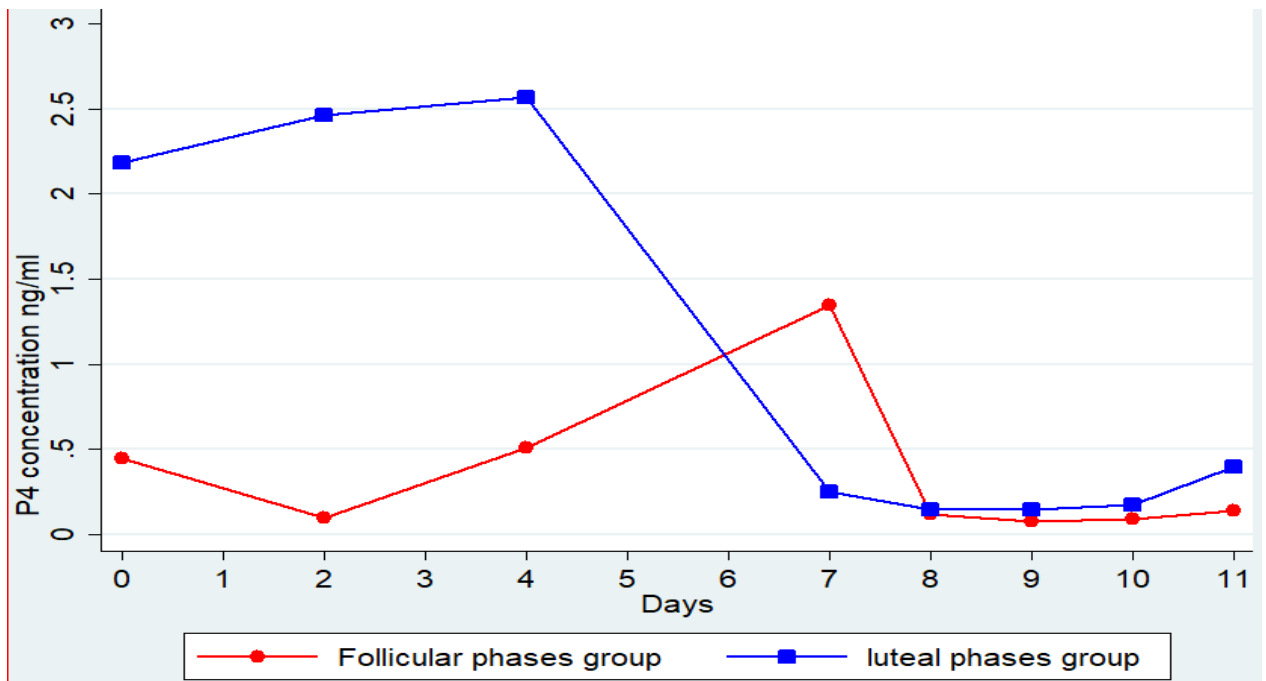


Figure 8: P4 concentration in the two groups of ewes'. As it is read from the figure at D2 of the 1st GnRH, the P4 was decreased in the follicular group and started to rise. At D7 (at PGF2 α), the

P4 reached its maximum and sharply decreased on D8 in the follicular group. In the luteal group, the P4 reaches its maximum at D4, and from that onwards, it is decreased.

4.2.4. Pattern of Change in Estrogen Concentration per Group

At D7 (at PGF2 α), the mean E2 concentration (15.48 \pm 0.87pg/ml) was significantly lower (P<0.05) in the luteal group than the follicular group (23.84 \pm 2.641pg/ml). In both groups, the Mean \pm SE E2 was elevated to the highest concentration on D8 (16.685 \pm 0.95pg/ml for luteal vs. 28.104 \pm 2.93pg/ml for follicular group, P<0.05) and dropped to the lowest concentration on D11 (13.502 \pm 2.4pg/ml for luteal vs. 20.078 \pm 2.40pg/ml for follicular, P<0.05). Behavioral estrus was observed when plasma E2 concentration levels reached maximum and when progesterone concentration was at its lowest.

Table 2: Results Plasma E2 Concentration in the follicular and luteal group ewes[?].

Days in experiment	Experimental groups			
	Follicular group		Luteal group	
	Plasma E2pg/ml Concentration		Plasma E2pg/ml Concentration	
	Mean \pm SE	(95% Conf. Interval)	Mean \pm SE	(95% Conf. Interval)
7	23.84 \pm 2.647	16.4-31.1	15.48 \pm 1.875	13.6 -19.7
8	28.108 \pm 2.93	19.9-36.2	16.685 \pm 0.959	9.5-21.4
9	23.546 \pm 2.682	16.1-30.9	14.085 \pm 1.679	7.2-20.3
10	23.028 \pm 1.442	19.02-27.03	13.815 \pm 2.065	5.6-21.3
11 ^{ϕ}	20.078 \pm 2.408	13.3-26.7	13.502 \pm 2.469	8.7-19.4

^{ϕ} = E2 concentration was not significantly different on day 11 (P>0.05). It was significantly higher (P<0.05) in follicular group from D7 to D10.

4.2.5 Pregnancy Rate

The proportion of ewes at standing estrus and mating was 100% for both the follicular and luteal groups. However, the pregnancy rate was significantly affected by treatment group and significantly lower in the follicular group (50%) than the luteal group (83.33%) ($P < 0.05$). After mating, the P4 concentration was significantly higher in the luteal phases groups of ewes on day 11 (0.399 ± 0.069 ng/ml) than in the follicular phases groups (0.141 ± 0.027 ng/ml) ($P < 0.05$), and the mean plasma E2 concentration on day 11 was at the highest level in the follicular phases than the luteal phases.

5. DISCUSSION

A thorough understanding of ovarian and hormonal alterations and their influences on the timing of estrus and ovulation is necessary to develop techniques that improve reproductive performance in sheep (Ali *et al.*, 2009). Estrus and ovulation synchronization are crucial components in assisted reproductive technology protocols, significantly enhancing reproductive function in ewes (Navanukraw *et al.*, 2014; Farrag, 2019).

Briefly, the following estrus signs were manifested by Menz ewes, either from natural or induced estrus. As ewes' estrus approached, ewes were frequently urinating and getting restless, Sniffing the ram's genital area and forequarters, running, frequently swinging the head back and looking at the ram's face, pushing the head into the ram's flank, and standing to be mounted were among many other signs. These findings were similar to previous reports (review in Talafha and Ababneh, 2011). However, the loss of appetite that was mentioned in many literature wasn't observed in Menz ewes when they were in estrus in this study.

The physical estrus signs observed include flagging tail rapidly, rapidly moving tail, vaginal discharge, vulva swelling, and reddening. During the standing estrus (sexual receptive time), the ewes vaginal secretion increases and more viscous events occur, which indicates the approach of the sexual active period in Menz ewes. Vaginal secretions in ewes are a significant source of signals pertaining to sexual attraction (Gatti and Ungerfeld, 2012, Ekiz *et al.*, 2013). Estrogens enter the systemic circulation and cause behavioral estrus by increasing vulva vascularity, causing swelling, reddening, mucus discharge, and vaginal wall thickening, preparing for copulation (Hafez and Hafez, 2013; Nakafeero, 2018; Habeeb and Kutzler, 2021).

The average estrus cycle length (15.46 ± 1.22 days), standing estrus duration (4.3 ± 0.53 hrs), and total estrus duration (35.3 ± 2.69 hrs) recorded in Menz breed ewes at this study were similar estrus cycle length and estrus duration to the previous studies (Hashemi *et al.*, 2006; Abecia *et al.*, 2011; Talafha and Ababneh, 2011; Zohara *et al.*, 2014; Arsoy and Sagmanlıgil, 2018; and Yadav *et al.*, 2021).

In this study, natural estrus lasted slightly longer than induced estrus. In natural estrus, the PGF2 α increases gradually, and natural CL luteolysis takes 72 hours to complete; however, in induced luteolysis, the PGF2 α picks up within a short time of administration, and luteolysis takes 6–24 hours and causes a more noticeable drop in plasma P4 concentrations (Cardenas *et al.*, 2004; Fierro *et al.*, 2013).

In this study, the estrus response rate in an induced estrus with a single PGF2 α injection was 63.64%. According to Talafha and Ababneh (2011) when PGF2 α is used as single dose it results in a 60% to 70% onset of estrus within 30–60h. When ewes were treated with double PGF2 α (Ayoub *et al.*, 2020) higher estrus responses (83.3%) than our study. The difference may be due to difference in breed, difference in animal's management and the environment. Single use PGF2 α has advantage of lower cost, and once-only handling ewes but PGF2 α is given to cyclic ewes, bred on estrus about one-third of ewes became non-response (Islam, 2011).

The luteolysis rate following PGF2 α treatment in the current study was 100% in follicular group and the P4 level was dropped to less than 0.5 ng/ml within 24h (on day 8) of PGF2 α . This was similar with 0.4 ng/ml reported by Ali *et al.*, (2009), and 0.45 ng/ml reported by Ashmawy (2011). Ali *et al.*, (2009) studied more than half of the ewes in GnRH-PGF2 α -GnRH group presented on the day of luteolysis without luteal structure. In the luteal groups of ewes, the CL was regressed spontaneously before PGF2 α and with the P4 concentration then increasing from day 10.

All ewes in the luteal group did not ovulate to D0 GnRH (Mean \pm SE P4 was 2.182 \pm 0.26 D0 GnRH and remains after D0 GnRH). Previous studies supported this. When GnRH is administered at higher plasma P4, there is no preovulatory LH surge or ovulation until PGF2 α is produced and causes luteolysis. Rather, the GnRH administered at the P4 level causes atresia of gonadotrophin-dependent large follicles (>4 mm in sheep) and allows follicular turnover and the new emergence of follicular waves (Goodman *et al.*, 1980; Campbell *et al.*, 1995; Leyva *et al.*, 1998; Gonzalez-Bulnes *et al.*, 2004; Ano-Perello *et al.*, 2020).

In the current study, based on the P4 concentration shift, the ovulation rate to D9 was 83.33% in the luteal and 100% follicular groups of ewes. In the luteal group of ewes, three ewes were ovulated between 24 and 48 hours of day 9 GnRH; however, in the follicular phase of ewes, three ewes were ovulated within the first 24 hours of day 9 GnRH. In Ali *et al.*'s (2009) study, ovulation started 48 hours after GnRH-PGF2 α -GnRH treatment. In a review of Al-Jaryan *et al.* (2023) and Talafha and Ababneh (2011), Awassi ewes with long estrus periods typically ovulate before the end of estrus, while those with shorter periods usually ovulate after the end of estrus. Estimating ovulation time is crucial for successful fertilization, especially in artificial insemination programs (Ekiz *et al.*, 2013).

In the present study, serum estradiol concentrations increased to 28.10 \pm 2.93 pg/ml on day 8 (within 24hr after PGF2 α treatment) in the follicular group and to 16.68 \pm 0.95 pg/ml on day 8 (again within 24hr after PGF2 α) in the luteal group. Previously, in the work of Talukder *et al.* (2018) and Letelier *et al.* (2011), estradiol concentration increased to its highest level before ovulation, peaking at follicular size, and then decreased to its basal level after ovulation. In the study of Campbell *et al.* (1990), it is reported that after PGF2 α injection, E2 secretion rates increase within 4–8 hours, then decline rapidly during the LH surge, reaching barely detectable levels within 16 hours. In both groups of ewes, the E2 concentration increased to the highest level within 24hrs after PGF2 α injection (on day 8), with signs of estrus behavior and it started to decline within 24hrs on day 9. In Takarkhede *et al.*'s (2014) study, the E2 concentration level was highest on the day of estrus but started declining after estrus.

In the current study, when the first GnRH (D0) was administered, the mean P4 concentration was 0.448 \pm 0.325ng/ml in the follicular group and 2.182 \pm 0.26ng/ml in the luteal group, and the mean P4 was increased until day 4 in both groups (0.508 \pm 0.073ng/ml in the follicular vs. 2.568 \pm 0.264ng/ml luteal groups). The mean P4 concentration at PGF2 α was decreased to 0.252 \pm 0.103 in the luteal group and increased to 1.348 \pm 0.165ng/ml in the follicular group.

The low P4 concentration at D0 GnRH in the follicular phase was due to the fact that the experiment at this phase was started at D16 of the estrus, which means the ewes had already undergone natural luteolysis at the estrus. Bazer (2020) and Al-Jaryan *et al.* (2023) indicated that diestrus was from day 4 to day 14 in ewes, and near the end of diestrus, progesterone receptors are down-regulated and luteolytic pulses of PGF2 α are produced to cause luteolysis. When PFG2 α was administered, the mean P4 was lower than the mid-luteal phase. This low P4 concentration also carried over to the pregnancy rate and caused a lower pregnancy rate.

The higher mean P4 at D0 GnRH in the luteal group was due to ewes in the diestrus stage of the estrus cycle (Arsoy and Sagmanlıgil, 2018; Bazer, 2020; Al-Jaryan *et al.*, 2023). However, in this group, when PGF2 α was given, the mean P4 was decreased as D7 (day of PGF2 α) was day 15 of estrus (proestrus). The onset of proestrus begins when the CL are fully regressed and the ovarian follicles begin producing significant amounts of estradiol-17 β (E2). A dominant follicle(s) is selected for ovulation, and the onset of estrus marks the beginning of the next estrous cycle (Bazer, 2020).

In the present study, the pregnancy rate was significantly ($P < 0.05$) higher in the luteal group than the follicular group. This effect was probably related to the fact that ewes in the luteal group had a greater P4 concentration before the onset of estrus and after mating than the follicular group ewes. In previous works, Ataman *et al.* (2013) indicated that low periovulatory progesterone secretion can lead to abnormal uterine function or poorly developed oocytes. The luteal phase provides the ewes with the most control due to its longer duration and increased responsiveness to manipulation (Yavuzer *et al.*, 2014).

Progesterone and estrogen play a crucial role in determining the proper function of the uterus for embryonic development and implantation (Ataman *et al.*, 2013). The reason for the higher pregnancy rate in the luteal phase's groups (83.33%) is that after ovulation, in the luteal phase's groups of ewes, the corpus luteum grows rapidly and starts to produce a significant amount of progesterone on day 11, compared to the follicular phase's groups of ewes. The concentration of progesterone increases as the CL increases in size during the early to mid-luteal phase (Vilarino *et al.*, 2013; Kaya *et al.*, 2017).

The E2 concentration was lower and the P4 concentration was higher in the luteal phase's group of ewes than in the follicular phases. The increase in progesterone prevents the synthesis of PGF2 α , providing pregnancy maintenance (Khan *et al.*, 2006; Ataman *et al.*, 2013), and that could be the reason for the higher pregnancy in the luteal group. Generally, many factors can affect pregnancy and estrous synchronization in sheep, which include protocols used, body composition, feed consumption, blood metabolites, hormonal response, CL responsiveness, and follicular development (Moonmanee and Yammuen-art, 2015; Maqhashu, 2019).

6. CONCLUSION AND RECOMMENDATION

Estrus event duration was similar between natural and induced estrus. Similarly, the behavioral and physical signs of estrus were also similar between natural and induced estrus. The pregnancy rate was significantly influenced by the phase of the estrus at the intuition of ovulation synchronization. Moreover, when the protocol was started on the 16th day of the estrus (follicular group), ewes were already ovulated, which led to an average plasma P4 level below mid luteal stage (1.348 ± 0.162 ng/ml) 7 days later (at PGF2 α), low pre-ovulatory P4, and a low pregnancy rate. When the protocol was started on the 8th day of the estrus (luteal group), ewes underwent spontaneous luteolysis 7 days later (at PGF2 α), although the protocol resulted in higher ovulation to D9 GnRH, higher pre-ovulatory P4, and a higher pregnancy rate.

Recommend the following, based on the findings:

- A detailed study should be done on the length of estrus stages (pro-estrus, estrus, met-estrus, and di-estrus), the pattern of corresponding hormonal dynamics per stage (P4, E2, FSH, LH pulse/surge), and the number of follicular waves throughout the estrus cycle of Menz ewes.
- In this study, observed estrus cycle lengths as short as 12 days and as long as 18 days. So to overcome the spontaneous ovulation and spontaneous luteolysis encountered in this experiment, recommend a study that assesses preferable days to start ovulation synchronization that would provide a better pregnancy rate in Menz breed ewes.

7. REFERENCES

- Abecia J.A., Forcada F. and González-Bulnes A. (2011): Pharmaceutical control of reproduction in sheep and goats. *Veterinary Clinics: Food Animal Practice*, **27**(1):67-79.
- Abecia J.A.; Forcada F. and Gonzalez-Bulnes A. (2012): Hormonal control of reproduction in small ruminants. *Animal Reproduction Science*. **130**:173–179
- Adams G.P., Matteri R.L., Kastelic J.P., Ko J.C.H. and Ginther, O. (1992): Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *Reproduction*, **94**(1), pp.177-188.
- Aderajew Tezera. (2015). Reproductive performance of local ewes and their response to prostaglandin based estrus synchronization protocol in south wollo zone, Amhara region (Doctoral dissertation, Bahir Dar University).
- Alavez R. A., Arroyo L. J., Montes P. R., Zamora B. R., Navarrete S. L. and Magaña S. H. (2014): Estrus synchronization using progestogens or cloprostenol in tropical hair sheep. *Tropical animal health and production*, **46**: 1515-1518.
- Ali A., Hayder M. and Saifelnaser E.O.H. (2009): Ultrasonographic and endocrine evaluation of three regimes for oestrus and ovulation synchronization for sheep in the subtropics. *Reproduction in Domestic Animals*, **44**(6), pp.873-878.
- Ali, A. (2007): Effect of time of eCG administration on follicular response and reproductive performance of FGA-treated Ossimi ewes. *Small Ruminant Research*, **72**(1), pp.33-37.
- Al-Jaryan I.L., AL-Thuwaini T.M., Merzah L.H. and Alkhammas A.H. (2023); Reproductive physiology and advanced technologies in sheep reproduction. *Reviews in Agricultural Science*, **11**, pp.171-180.
- Amarantidis I., Karagiannidis A., Saratsis P.H. and Brikas, P. (2004): Efficiency of methods used for estrous synchronization in indigenous Greek goats. *Small Ruminant Research*, **52**(3), pp.247-252.
- Amiridis G.S. and Cseh S., (2012). Assisted reproductive technologies in the reproductive management of small ruminants. *Animal reproduction science*, **130**(3-4), pp.152-161.
- Ano-Perello A., Santos-Jimenez Z., Encinas T., Martinez-Ros P. and Gonzalez-Bulnes A. (2020): Use of GnRH for synchronization of the follicular wave in assisted reproductive technologies in sheep: *A preliminary study*. *Animals*, **10**(7), p.1208.

- Arsoy D. and Sağmanlıgil V. (2018): Reproductive cycles in white Karaman ewes: comparison of ovarian hormone secretion and reproductive behavior in non-pregnant and pregnant ewes in semi-intensive conditions. *Acta Scientiarum. Animal Sciences*, **40**, p.e39908.
- Ashmawy T.A.M. (2011). Timing ovulation in ewes treated with ovsynch protocol by different times of pgf2 α injection during the breeding season 23-30.
- Ataman M.B., Aköz M. and Akman O. (2006): Induction of synchronized oestrus in akkaraman cross-bred ewes during breeding and anestrus seasons: the use of short-term and long-term progesterone treatments, *Revue Med. Vet.* **157**(5): 257-260.
- Ataman M.B., Aköz, M., Saribay, M.K., Erdem, H. and Bucak, M.N. (2013): Prevention of embryonic death using different hormonal treatments in ewes. *Turkish Journal of Veterinary & Animal Sciences*, **37**(1), pp.6-8.
- Ayoub M.A., Tharwat R.A.M., Yaseen M.A. and Soliman E.S. (2020): Using Progesterone and Prostaglandin F2 α for Ewes Estrus Synchronization during Summer Season in Egypt. *Journal of Animal, Poultry & Fish Production*, **9**(1), pp.1-8.
- Barrett D.M. (2002). An investigation of conventional oestrous synchronization techniques in the ewe (Doctoral dissertation, Thesis. University of Saskatchewan, Saskatoon, Saskatchewan, Canada. pp **151**).
- Bartlewski P.M., Baby T.E. and Giffin J.L. (2011): Reproductive cycles in sheep. *Animal reproduction science*, **124**(3-4):pp.259-268.
- Bazer F.W. (2020). Reproductive physiology of sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*). *Animal Agriculture*, pp.**199**-209.
- Bekahegn Wondim. (2019). Estrus Synchronization and Artificial Insemination Technologies in Abergelle Goat at Station and on-farm Conditions of Waghemira Zone, Ethiopia (Doctoral dissertation).
- Besufkad Shanbel, Betsha Simret, Demis Chekol, Zewude Tesfaye, Rouatbi M, Getachew, Tesfaye, Haile Aynalem, Rischkowsky B. and Rekik, M. (2020): Field synchronization of Ethiopian highland sheep for fixed time artificial insemination: Improvement of conception rate with a double injection of prostaglandin at 11 days. *Journal of Applied Animal Research*, **48**(1), pp.413-418.
- Biehl M.V., de Ferraz Junior M.V.C., Barroso J.P.R., Susin I., Ferreira E.M., Polizel D.M. and Pires, A.V. (2019): The reused progesterone device has the same effect on short or long

- estrus synchronization protocols in tropical sheep. *Tropical animal health and production*, **51**, pp.1545-1549.
- Blache D and Martin GB. (2005): Neural and endocrine mechanisms underlying the synchrony of sexual behaviour and ovulation in the sheep. *Oxf Rev Reprod Biol*, **17**:205–54.
- Boscós C.M., Samartzi F.C., Lymberopoulos A.G., Stefanakis A. and Belibasaki S. (2003): Assessment of progesterone concentration using enzymeimmunoassay, for early pregnancy diagnosis in sheep and goats. *Reproduction in Domestic Animals*, **38**(3), pp.170-174.
- Boscós C.M., Samartzi, F.C., Dellis, S., Rogge, A., Stefanakis, A. and Krambovitis, E., (2002): Use of progestagen–gonadotrophin treatments in estrus synchronization of sheep. *Theriogenology*, **58**(7), pp.1261-1272.
- Campbell B.K., Mann G.E., McNeilly A.S. and Baird D.T. (1990): The pattern of ovarian inhibin, estradiol, and androstenedione secretion during the estrous cycle of the ewe. *Endocrinology*, **127**(1), pp.227-235.
- Campbell B.K.; Scaramuzzi R.J.; and Webb R. (1995): Control of follicle development and selection in sheep and cattle. *J. Reprod. Fertil. Suppl*, **49**, 335–350.
- Cardenas H., Wiley T.M. and Pope W.F. (2004): Prostaglandin F_{2α}-induced estrus in ewes exhibiting estrous cycles of different duration. *Theriogenology*, **62**(1-2):123-129.
- Contreras-Solis I., Vasquez B., Diaz T., Letelier, C. Lopez-Sebastian A. and Gonzalez-Bulnes, A. (2009): Efficiency of estrous synchronization in tropical sheep by combining short-interval cloprostenol-based protocols and “male effect”. *Theriogenology*, **71**(6), pp.1018-1025.
- CSA (2021). Agricultural sample survey volume ii, report on livestock and livestock characteristics.
- Cueto, M., Gibbons, A., Alberio, R., Taddeo, H. and Gonzalez-Bulnes, A. (2006): Timing of emergence of ovulatory follicles in polyovulatory goats. *Animal reproduction science*, **91**(3-4), pp.275-284.
- Daly J., Smith H., McGrice H.A., Kind, K.L. and Van Wettere, W.H. (2020): Towards improving the outcomes of assisted reproductive technologies of cattle and sheep, with particular focus on recipient management. *Animals*, **10**(2), p.293.
- Dardente H., Lomet D., Robert, V., Decourt C., Beltramo M. and Pellicer-Rubio, M.T. (2016): Seasonal breeding in mammals: From basic science to applications and back. *Theriogenology*, **86**(1), pp.324-332.

- De Freitas A.C.B., Vega W.H.O., Quirino C.R., Junior A.B., David C.M.G., Geraldo A.T., Rua, M.A.S., Rojas L.F.C., de Almeida Filho J.E. and Dias A.J.B. (2018): Surface temperature of ewes during estrous cycle measured by infrared thermography. *Theriogenology*, **119**:245-251.
- Delgadillo J.A., Gelez H., Ungerfeld R., Hawken P.A. and Martin G.B. (2009): the 'male effect' in sheep and goats revisiting the dogmas. *Behavioural brain research*, **200**(2), pp.304-314.
- Deligiannis C., Valasi I., Rekkas C.A., Goulas P., Theodosiadou E., Lainas T. and Amiridis G.S., (2005): Synchronization of ovulation and fixed time intrauterine insemination in ewes. *Reproduction in domestic animals*, **40**(1), pp.6-10.
- Dias L.M.K., Sales, J.N.S., Viau, P., Barros, M.B.P., Nicolau, S.S., Simões, L.M.S., Alves, N.G., Alonso, M.A., Valentim, R. and Oliveira C.A.D. (2018): Although it induces synchronized ovulation, hCG reduces the fertility of Santa Ines ewes submitted to TAI. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **70**, pp.122-130.
- Driancourt M.A., Gibson W.R. and Cahill, L.P. (1985): Follicular dynamics throughout the oestrous cycle in sheep. A review. *Reproduction Nutrition Development*, **25**(1A), pp.1-15.
- Ekiz EE, Ekiz B and Koçak O. (2013): Effects of ram presence during synchronization period and previous experience on certain estrus parameters and sexual behaviors in Kivircik ewes. *Turkish J Vet Anim Sci*; **37**:189–93.
- Faigl V., Vass N., Javor A., Kulcsar M., Solti, L., Amiridis, G. and Cseh, S. (2012): Artificial insemination of small ruminants A review. *Acta Veterinaria Hungarica*, **60**(1), pp.115-129.
- Fair S., Hanrahan J.P., O'Meara C.M., Duffy P., Rizos D., Wade M., Donovan A., Boland M.P., Lonergan P. and Evans, A.C.O. (2005): Differences between Belclare and Suffolk ewes in fertilization rate, embryo quality and accessory sperm number after cervical or laparoscopic artificial insemination. *Theriogenology*, **63**(7), pp.1995-2005.
- Farrag B. (2019). Productive characteristics and reproductive responses to estrus synchronization and flushing in Abou-Delik ewes grazing in Arid Rangelands in Halaieb-Shalateen-Abouramad Triangle of Egypt. *World's Veterinary Journal*, **9**(3), pp.201-210.
- Fierro S., Gil J., Vinales C. and Olivera-Muzante J. (2013): the use of prostaglandins in controlling estrous cycle of the ewe: A review. *Theriogenology*, **79**(3), pp.399-408.

- Fierro S., Viñoles C. and Olivera-Muzante J. (2016): Concentrations of steroid hormones, estrous, ovarian and reproductive responses in sheep estrous synchronized with different prostaglandin-based protocols. *Animal reproduction science*, **167**, pp.74-82.
- Gatti M. and Ungerfeld R. (2012): Intravaginal sponges to synchronize estrus decrease sexual attractiveness in ewes. *Theriogenology*, **78**(8), pp.1796-1799.
- Gebrekidan Berihu., Gebremeskel Gebremariam., Weldegebrial Bahlibi and Asfaw Yohanes., Tekele (2014): Efficacy of GnRH-PGF 2α Treatment in Synchronization of Estrus in Ethiopian Local Sheep Breed. *International Journal of Livestock Research*, **4**(7), pp.40-48.
- Getachew Tesfaye. (2008). Characterization of Menz and Afar indigenous sheep breeds of smallholders and pastoralists for designing community-based breeding strategies in Ethiopia (Doctoral dissertation, Haramaya University).
- Gidena A. (2017). Effect of estrus and ovulation synchronization protocols in local goats during the breeding season (Doctoral dissertation, Mekelle University).
- Girmay Aderajew Mekonnen, Alemu Zinabu Assefa, Mengesha Sisay Derso, Dinssa Daniel Abera, Wagari Bedasa, Weldegebriel Mesaye Getachew, Serte Melaku Gizaw, Tsigereda Assefa Alemayehu , Kenea Moa Abate, Weldetinsae Abel. and Teklu Kirubel Tesfaye. (2022): Effect of demographic disparities on the use of the JMP ladders for water, sanitation, and hygiene services in Bishoftu Town, Ethiopia. *Discover Water*, **2**(1), p.8.
- Gizaw Solomon., Tesfay Yayneshet., Mekasha Yoseph., Mekuriaw Zeleke., Gugsu Tadesse., Ebro Abule., Gebremedhin Berhanu., Hoekstra Dirk and Tegegne Azage. (2016): Hormonal oestrus synchronization in four sheep breeds in Ethiopia: impacts on genetic improvement and flock productivity. Lives Working Paper.
- Gizaw Solomon, Van Arendonk, Johan AM, Komen, Hans, Windig and Hanotte, Olivier. (2007): Population structure, genetic variation and morphological diversity in indigenous sheep of Ethiopia. *Animal Genetics*, **38**(6), pp.621-628.
- Gonzalez-Bulnes A., Baird D.T., Campbell B.K., Cocero M.J., García-García R.M., Inskoop E.K., López-Sebastián A., McNeilly A.S., Santiago-Moreno J., Souza C.J. and Veiga-Lopez, A. (2004): Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. *Reproduction, fertility and Development*, **16**(4), pp.421-435.
- Gonzalez-Bulnes A., Diaz-Delfa C., Garcia-Garcia R., Urrutia B., Carrizosa J. & Lopez-Sebastian, A. (2005): Origin and fate of preovulatory follicles after induced luteolysis at different

- stages of the luteal phase of the oestrous cycle in goats. *Animal Reproduction Science*, **86**, 237-245.
- Goodman R.L. and Karsch F.J. (1980): Pulsatile secretion of luteinizing hormone: differential suppression by ovarian steroids. *Endocrinology*, **107**(5), pp.1286-1290.
- Habeeb H.M.H. and Kutzler M.A. (2021): Estrus synchronization in the sheep and goat. *Veterinary Clinics: Food Animal Practice*, **37**(1), pp.125-137.
- Hafez E.S.E. and Hafez, B. eds. (2013): Reproduction in farm animals. John Wiley & Sons.
- Hashemi M., Safdarian M. and Kafi, M. (2006): Estrous response to synchronization of estrus using different progesterone treatments outside the natural breeding season in ewes. *Small Ruminant Research*, **65**(3), pp.279-283.
- Islam R. (2011). Synchronization of estrus in cattle: a review. *Veterinary World*, **4**(3).
- Jainudeen M.R., Wahid, H. and Hafez, E.S.E. (2000): Sheep and goats. *Reproduction in farm animals*, pp.172-181.
- Johnson S.K., Dailey R.A., Inskeep E.K. and Lewis P.E. (1996): Effect of peripheral concentrations of progesterone on follicular growth and fertility in ewes. *Domestic Animal Endocrinology*, **13**(1), pp.69-79.
- Kaya S., Kaçar C., Polat B., Çolak A., Kaya D., Gürcan I.S., Bollwein H. and Aslan S. (2017): Association of luteal blood flow with follicular size, serum estrogen and progesterone concentrations, and the inducibility of luteolysis by PGF2 α in dairy cows. *Theriogenology*, **87**, pp.167-172.
- Khan T.H., Beck N.F., Mann G.E. and Khalid M. (2006): Effect of post-mating GnRH analogue (buserelin) treatment on PGF2 α release in ewes and ewe lambs. *Animal reproduction science*, **95**(1-2), pp.107-115.
- Laghrou W., Safsaf B., Alleg, N., Tlidjane M. and Ouarest, A. (2020): Effect of feeding different ratios of concentrate roughage during induced estrus on hormonal and reproductive performances of ewes. *Development*, **32**(3).
- Lehloeny K.C., Greyling J.P.C. and Schwalbach, L.M.J. (2005): Reproductive performance of South African indigenous goats following oestrous synchronization and AI. *Small Ruminant Research*, **57**(2-3), pp.115-120.
- Leta Samson and Frehiwot Mesele. (2014): spatial analysis of cattle and shoat population in Ethiopia: growth trend, distribution and market access. *Springer Plus*, **3**(1), pp.1-10.

- Letelier C.A., Contreras-Solis I., García-Fernández R.A., Sánchez M.A., García-Palencia P., Sánchez B., Ariznavarreta C., Tresguerres J.A.F., Flores J.M. and Gonzalez-Bulnes A. (2011): Effects of oestrus induction with progestagens or prostaglandin analogues on ovarian and pituitary function in sheep. *Animal reproduction science*, **126**(1-2), pp.61-69.
- Leyva V., Buckrell B.C. and Walton, J.S. (1998): Regulation of follicular activity and ovulation in ewes by exogenous progestagen. *Theriogenology*, **50**(3), pp.395-416.
- Liu T.C., Chiang C.F., Ho, C.T. and Chan J.P.W. (2018): Effect of GnRH on ovulatory response after luteolysis induced by two low doses of PGF2 α in lactating dairy cows. *Theriogenology*, **105**, pp.45-50.
- Maqhashu A., (2019). Characterization and evaluation of reproductive performance in Bapedi sheep breed (Doctoral dissertation, University of the Free State).
- Martin G.B., Milton J.T.B., Davidson R.H., Hunzicker G.B., Lindsay D.R. and Blache D. (2004): Natural methods for increasing reproductive efficiency in small ruminants. *Animal reproduction science*, **82**, pp.231-245.
- Martinez M. F., Adams G. P., Kastelic J. P., Bergfelt D. R., and Mapletoft R. J. (2000): Induction of follicular wave emergence for estrus synchronization and artificial insemination in heifers. *Theriogenology*, **54**:757-769.
- Menchaca A., dos Santos-Neto P.C., Cuadro F., Souza-Neves M. and Crispo, M. (2018): From reproductive technologies to genome editing in small ruminants: An embryo's journey. *Animal Reproduction*, **15**(Suppl 1), p.984.
- Moonmanee T. and Yammuen-art S., (2015). Relationships among feed intake, blood metabolites, follicle size and progesterone concentration in ewes exhibiting or not exhibiting estrus after estrous synchronization in the tropics. *Agriculture and Agricultural Science Procedia*, **5**, pp.151-158.
- Nakafeero A. (2018). Response to Different Oestrous Synchronisation Protocols and Fertility of Ewes Following Artificial Insemination. University of Pretoria (South Africa).
- Navanukraw C., Khanthusaeng V., Kraison A. and Uriyapongson S. (2014): Estrous and ovulatory responses following cervical artificial insemination in Thai-native goats given a new or once-used controlled internal drug release with human chorionic gonadotropin. *Tropical animal health and production*, **46**, pp.1441-1446.

- Rekik M., Haile A., Abebe A., Muluneh D., Goshme S., Ben Salem I., Hilali M.E.D., Lassoued N., Chanyalew Y. and Rischkowsky, B. (2016): GnRH and prostaglandin-based synchronization protocols as alternatives to progestogen-based treatments in sheep. *Reproduction in domestic animals*, **51**(6), pp.924-929.
- Ricciotti E and FitzGerald GA. (2011): "Prostaglandins and inflammation". *Arteriosclerosis, Thrombosis, and Vascular Biology*, **31** (5): 986–1000
- Rosa H.J. and Bryant M.J. (2003): Seasonality of reproduction in sheep. *Small ruminant research*, **48**(3), pp.155-171.
- Rosasco S.L., Beard J.K., Hallford D.M. and Summers A.F. (2019): Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability. *Animal reproduction science*, **210**, p.106191.
- Seekallu S.V., Toosi, B.M., Duggavathi, R., Barrett D.M.W., Davies K.L., Waldner, C. and Rawlings, N.C. (2010): Ovarian antral follicular dynamics in sheep revisited: Comparison among estrous cycles with three or four follicular waves. *Theriogenology*, **73**(5), pp.670-680.
- Shabankareh H.K., Habibizad, J. and Torki, M. (2009); Corpus luteum function following single and double ovulation during estrous cycle in Sanjabi ewes. *Animal reproduction science*, **114**(4), pp.362-369.
- Takarkhede R.C., Honmode J.D. and Kadu M.S. (2014): Seasonal variations in estradiol-17 β and progesterone level during oestrus cycle in Malpura ewes. *The Indian Journal of Animal Sciences*, **72**(4).
- Takele Moges. (2016). Phenotypic characterization of indigenous sheep and farmers' breeding practices in south wollo zone, Amhara region (Doctoral dissertation, Bahir Dar University).
- Talafha A.Q. and Ababneh, M.M. (2011). Awassi sheep reproduction and milk production. *Tropical animal health and production*, **43**, pp.1319-1326.
- Talukder M.R.I., Hasan M., Rosy T.A., Bari F.Y. and Juyena N.S., (2018): Monitoring vaginal electrical resistance, follicular waves, and hormonal profile during oestrous cycle in the transition period in Bangladeshi sheep. *Journal of Veterinary Research*, **62**(4), pp.571-579.
- Talukder S., Thomson PC., Kerrisk KL., Clark CEF., Celi P. (2015): Evaluation of infrared thermography body temperature and collar-mounted accelerometer and acoustic

- technology for predicting time of ovulation of cows in a pasture-based system. *Theriogenology*; **83**:739–48.
- Tibbo M. (2006). Productivity and health of indigenous sheep breeds and crossbreeds in central Ethiopian Highlands (Doctoral dissertation, Swedish University of Agricultural Sciences).
- Titi HH, Kridli RT. and Alnimer MA. (2010): Estrus Synchronization in Sheep and Goats Using Combinations of GnRH, Progestagen and Prostaglandin F 2 α . *Reproduction in Domestic Animals*, **45**(4):594-9.
- Viana J.H.M., Ferreira A.M., SA, W.F. and Camargo L.S.A. (2000): Follicular dynamics in Zebu cattle. *Pesq. Agropec. Bras.* **35**:2501–2509.
- Vilarino M., Cuadro F., dos Santos-Neto P.C., García-Pintos, C. and Menchaca, A. (2017): Time of ovulation and pregnancy outcomes obtained with the prostaglandin-based protocol Synchrovine for FTAI in sheep. *Theriogenology*, **90**, pp.163-168.
- Vilarino M., Rubianes E. and Menchaca A. (2013): Ovarian responses and pregnancy rate with previously used intravaginal progesterone releasing devices for fixed-time artificial insemination in sheep. *Theriogenology*, **79**(1), pp.206-210.
- Wang H., Feng, X., Muhatai, G. and Wang, L. (2022): Expression profile analysis of sheep ovary after superovulation and estrus synchronisation treatment. *Veterinary Medicine and Science*, **8**(3): 1276-1287.
- Wei S., Chen S., Wei B., Liu, Z., Bai T. and Lin J. (2016): Estrus synchronization schemes and application efficacies in anestrus lanzhou fat-tailed ewes. *Journal of Applied Animal Research*, **44**(1), pp.466-473.
- Whitley N.C. and Jackson, D.J. (2004). An update on estrus synchronization in goats: a minor species. *Journal of animal science*, **82**(suppl_13), pp.E270-E276.
- Yadav V., Saini G., Pandey A.K. and Kumar, S. (2021): Neuro-endocrine mechanisms controlling reproductive seasonality in sheep: a review. *Pharm Innov J*, **10**, pp.160-5.
- Yavuzer Ü, Aral F., Zonturlu A.K., Korkmaz Ö. and Emre B. (2014): Effect of Recombinant Luteinizing Hormon (rLH) on Some Fertility Parameters in Awassi Ewes Synchronized with PGF2 α in the Breeding Season. *Harran niv Vet FakDerg*, **3**(1), pp.6-12.
- Zohara B.F., Islam M.F., Alam M.G.S. and Bari F.Y., (2014): Exfoliative vaginal cytology and serum progesterone during the estrous cycle of indigenous ewes in Bangladesh. *Journal of Embryo Transfer*, **29**(2), pp.183-188.

8. APPENDIX

Annex 1: Age determination procedures

- 1 Year (12-18 Months)
The central pair of milk teeth is replaced by larger, permanent incisors (first pair of permanent incisors). The sheep will have two larger front teeth surrounded by smaller milk teeth.
- 2 Years (18-24 Months)
The second pair of milk teeth (next to the central pair) is replaced by permanent incisors. The sheep now has four permanent incisors.
- 3 Years
The third pair of milk teeth is replaced by permanent incisors. The sheep will have six permanent incisors.
- 4 Years:
The fourth and final pair of milk teeth is replaced by permanent incisors, giving the sheep a full set of eight permanent incisors.
- 4-6 Years
The teeth are now fully erupted and align in a straight row. The incisors are broad and have a more worn appearance.
- 6-10 Years:
The teeth show significant wear, becoming rounded or even gapped. The wear becomes more pronounced as the sheep ages.
- 10+ Years

The teeth can become very worn down, broken, or may even fall out. The gums may recede, and the teeth can become loose.

Annex 2: E2 and P4 Analysis procedures

a) Preparation:

- ✓ Ensure that the Cobas 6000 analyzer is properly calibrated and all reagents are at the appropriate temperature.
- ✓ Gather all necessary materials including Ewes plasma samples, reagents, calibrators, and controls.

b) Sample Preparation:

- ✓ Label sample tubes with unique identifiers.
- ✓ Mix the sheep plasma samples by gentle inversion.
- ✓ Pipette the required volume of each sheep plasma sample into the labeled tubes.

c) Assay Setup:

- ✓ Follow the manufacturer's instructions to prepare the Cobas 6000 system for the E2 and P4 assays.
- ✓ Load reagents, calibrators, controls, and sample tubes onto the instrument according to the system's protocol.

d) Instrument Operation:

- ✓ Initiate the analysis on the Cobas 6000 system, selecting the appropriate assay methods for E2 and P4.
- ✓ The system will automatically process the samples, perform the necessary reactions, and measure the resulting absorbance or fluorescence.

e) Data Analysis:

- ✓ Once the analysis is complete, review the results generated by the Cobas 6000 system for E2 and P4 levels in each ewes plasma sample.

Annex 3: Transrectal Ultrasonography procedures

- ewes in a standing position Fecal pellets were removed, digitally
- Gel inserted with a syringe into the rectum to act as a coupling medium between the rectal wall and transducer.
- The transducer inserted and manipulated in the rectum by external control of the extension.
- The urinary bladder, cranial vagina, and cervix were viewed in the longitudinal planes while the transducer is being inserted.
- After the cervix and caudal uterus are viewed.

Annex 4: Blood Collection Procedure

- Restrain animal with head elevated and jugular vein exposed
- Clip (optional) a small area over the jugular groove, and swipe with antiseptic gauze to remove superficial dirt and debris.
- The area of blood collection site will be cleaned and disinfected with the soaked cotton swab to minimize the contamination.
- . Occlude jugular vein by applying pressure at the base of the jugular groove and visualize raised vein
- Insert needle through skin and into vein at 20° angle.
- Once the needle inserted, stabilize needle and push the vacutainer tube into hub.
- Once collection complete, release pressure to the vein then, applying pressure over injection site with gauze, remove needle.
- Dispose of needle in approved Sharps container.
- After collection, the blood samples will be labeled and brought to the laboratory

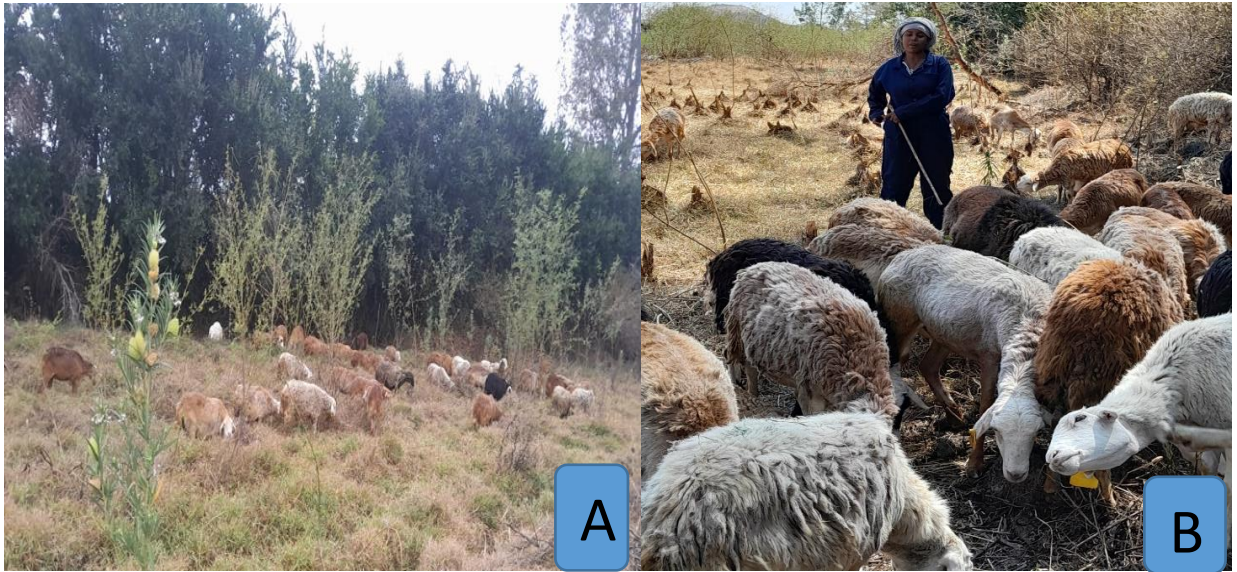
Annex 5: Ram vasectomy procedure

The procedure will be done by Vet. Surgeon.

- The surgeons will be dressed in protective clothing.
- Restraining (the sheep will be held in a sitting position).
- Local anesthesia (lidocaine) will be given at the surgical site. A ring block around the cord can help with analgesia.
- The scrotum will be clipped (if needed) and scrubbed, disinfect at the surgical site.
- A clean towel or sterile drape will be placed under the scrotum for suture management.
- Identify the spermatic cord in the neck of the scrotum.
- The vas deferens is located on the axial side.
- Will make a longitudinal incision over the axial side of the cord through the skin and subcutaneous tissues.
- Elevate the cord onto the Kelly forceps to keep it stable and be able to see the white vas deferens on the side through the wall of the tunic.
- Incise the tunic that covers the structures within.
- Once the tunic is open, find the firmer structure that is somewhat isolated.
- Elevate this on the mosquito hemostat. When relaxed, the vas deferens has a slightly coiled appearance to it.
- Clamp the ends of the section of vas deferens to be removed.
- Ligate each end to prevent sperm granuloma formation and remove at least 1 cm of section.
- The skin will be closed routinely, and 2% tincture iodine will be applied to the incision line to prevent infection.
- Postoperatively, antibiotics will be given for three days.
- Everyday Monitoring the wound for signs of infection to prevent and separate it from other sheep.
- Avoid using the ram to access females until the wound recovers

Annex 6: Different kinds of images were taken during the experiment.

Plate 1: grazing period during the day and night.



A, B) Grazing the daytime



C) Grazing the daytime

D) Feeding during the night

Plate 2: picture of Ewes' physical and behavioral estrus signs observed during the experiment in Menz breed ewes.



A) The rams preparing the ewe

B) standing to be mount



C) Mounting to other ewe

D) more than two ewes in estrus (induced estrus)



E) Flaggering tails

F) sniffing anogenital area

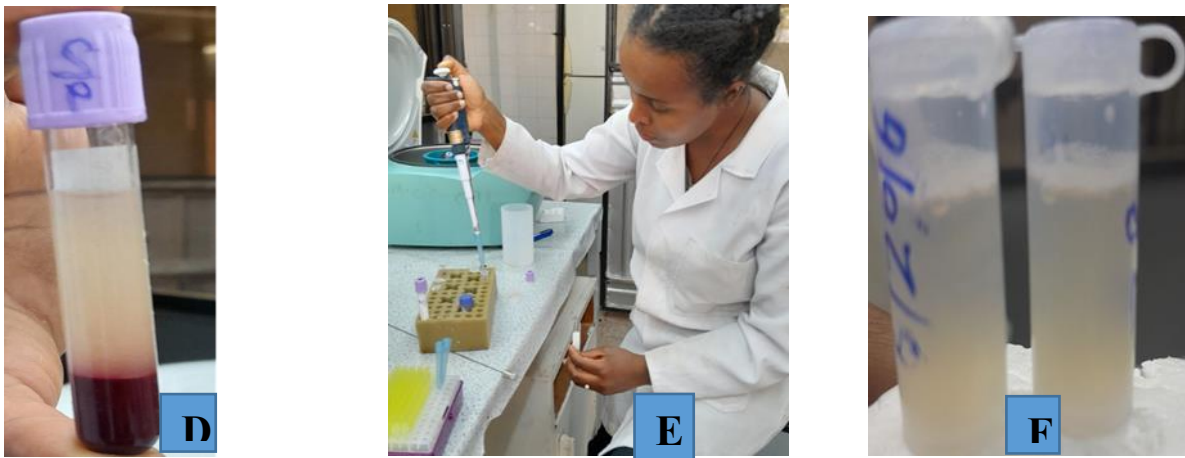
Plate 3: pictures of blood collection and blood plasma separation procedures



A) Blood collecting from jugular vein

B) Labeling the sample

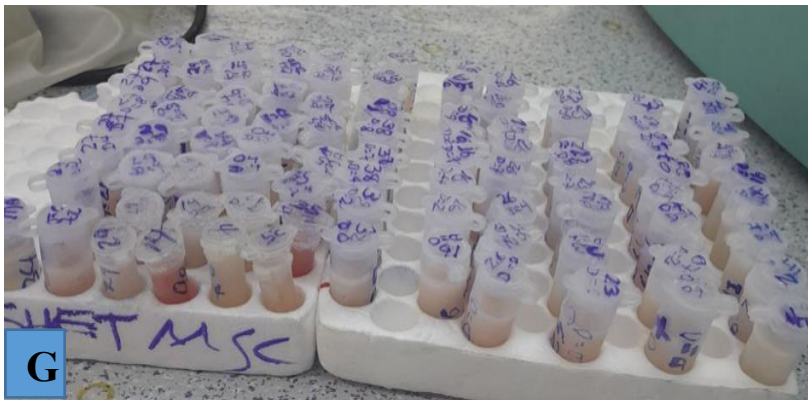
C) Collected blood



D) Blood plasma after Centrifuge

E) transferring into plastic 2-ml tubes

F) pure plasma sample



G) Collected plasma samples from two groups

Plate 4: Tran’s rectal pregnancy diagnosis



A) Trans-rectal pregnancy diagnosis in ewes **B)** Placentomes were detected as small grey O-shaped structures around the edges of the fluid-filled vesicle. **C)** Presence of a fluid-filled uterine horn.

Annex 7: Ethical Clearance

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 ADDIS ABABA UNIVERSITY
 College of Veterinary Medicine and Agriculture
 Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/02/26/16/2024

Name of Applicant: **Marshet Netsere Woldeyes (BSc, MSc student)**

Address: Department Clinical Studies, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Estrus characteristics of Menz ewes and effect of estrus cycle phases at initiation of ovulation synchronization on pregnancy rate*

Date of application: **December, 2023**
 Nature of the project: **Experimental study**
 Target animal species: **Sheep**
 Number of animals involved: **15**
 Study area: **CVMA-Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/02/16/024, 26/03/2024**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of: **Marshet Netsere Woldeyes.**

Professor Getachew Terefe (DVM, PhD)
 Chairman Signature

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