



**Evaluation of Artificial Insemination in Menz Sheep Following Estrus
Synchronization with Progestogen and Prostaglandin-based
Synchronization Protocols**

MSc Thesis

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College of Agriculture

Hawassa, Ethiopia

March, 2019

**Evaluation of Artificial Insemination in Menz Sheep Following Estrus
Synchronization with Progestogen and Prostaglandin-based
Synchronization Protocols**

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A Thesis Submitted to School of Animal and Range Sciences

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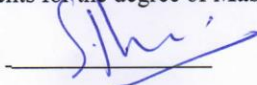
**In Partial Fulfillment of the Requirements for the Degree of Master of
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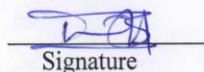
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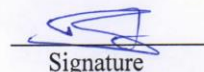
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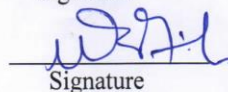
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DEDICATION

I dedicated this thesis manuscript to my most respected and beloved father Besufkad Yeshaw, and my mother Shitaw Tristewold for nursing me with affection and love and for their dedicated parenthood to be successful in life.

STATEMENT OF THE AUTHOR

I declare that this thesis is my original work and all sources of materials used for this thesis have been duly acknowledged. This thesis is submitted in partial fulfillment of the requirement for degree of Master at Hawassa University and placed at the University, DBARC, ICARDA and EIAR libraries to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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ABBREVIATIONS AND ACRONYMS

AI	Artificial insemination
AV	Artificial vagina
BCS	Body condition score
BOKU	the Austrian University of Natural Resources and Applied Sciences
CBBP	Community based breeding program.
CIDR	Controlled internal drug release
CL	Corpus luteum
DBARC	Debre Birhan Agricultural Research Center
EIAR	Ethiopian Institute of Agricultural Research
FGA	Fluorogestone acetate
FSH	Follicular stimulating hormone
GnRH	Gonadotrophic releasing hormone
ICARDA	International Center for Agricultural Research in the Dry Areas
ILRI	International Livestock Research Institute
LH	Luteinizing hormone
LIVES	Livestock and Irrigation Value chains for Ethiopian Smallholders
LSM	Least square means
MGA	Melengestrol acetate
NARS	National Agricultural Research System
PGF2 α	Prostaglandin F2alpha
PMSG	Pregnant mare's serum gonadotropin
SE	Standard error

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Evaluation of Artificial Insemination in Menz Sheep Following Estrus Synchronization with Progestogen and Prostaglandin-based Synchronization Protocols

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ABSTRACT

Village based genetic improvement programs can't be effective if lambing is not planned. If lambing is thinly distributed across the year it will expose most of the lambs to unfavorable seasons for both survival and growth. To mitigate these problems, strategies that controls the time of lambing should be thought. To this end, the present study evaluated success of estrus synchronization and artificial insemination in Menz sheep. For this, pure and cross breed (Dorper x Menz) experiments were conducted in Menz community based breeding program villages and Debre Birhan Agricultural Research Center respectively. For pure breeding a total of 166 Menz ewes were selected from Debre Birhan Agricultural Research Center and community based breeding program villages and allocated to one of the three protocols; Protocol 1 (n=60) single injection of PGF2 α ; Protocol 2 (n=54) double injection of PGF2 α 7 days apart; and Protocol 3 (n=52) double injection of PGF2 α 11 days apart. For cross breeding a total of 83 Menz ewes were kept in Debre Birhan Agricultural Research Center and assigned to one of the four protocols; Protocol 1 (n=20) single injection of PGF2 α ; Protocol 2 (n=24) double injection of PGF2 α 7 days apart; Protocol 3 (n=19) double injection of PGF2 α 11 days apart; and Protocol 4 (n=20) intravaginal progestagen sponges, followed by injection of PMSG at sponge withdrawal. Fixed time cervical insemination was performed in estrous ewes 48-51 hr following last PGF2 α and PMSG injection with 0.25 ml fresh diluted Menz and Dorper semen. The effect of synchronization protocols, body condition, parity, management and semen genotype on the oestrous response, pregnancy, lambing and fecundity rates of ewes were analyzed using nonparametric tests. The effect of synchronization protocols, semen genotype, body condition, parity and sex of lambs on the litter size and litter weight were analyzed using GLM of SAS (9.0). The same statistical procedures were adopted to analyze the effect of genotype and ejaculation frequency on semen quality parameter (volume, mass motility and concentration). Out of 249 ewes synchronized 77.5% of ewes exhibited overt sign of oestrous within 48 hr of hormone injection. There was no significant difference in percentage of ewes exhibiting overt signs of oestrous across protocols, parity and management. However, ewes that had between 2.5 and 3.0 body condition scores were significantly ($p<0.05$) higher responsive to hormonal treatment compared to ewes that had 2.0 body conditions score (80.7 vs 55.0%). The overall pregnancy, lambing and fecundity rates were 59.1, 58.0, and 98.2% respectively. There were no significant differences in pregnancy, lambing, and fecundity rates between ewes due to differences in synchronization protocols, body condition, and parity. On the other hand ewes inseminated with Dorper semen had significantly ($p<0.05$) higher pregnancy and lambing rates as compared to ewes inseminated with Menz. In general the current work indicated that oestrous could be effectively synchronized in Menz sheep. Thus artificial insemination could be an area of intervention in sheep genetic improvement programs.

Key words: Artificial Insemination, Menz, PGF2 α , Progestogen, Synchronization

1. INTRODUCTION

1.1. Background and Justification

Small ruminant production is a major component of the livestock sector in Ethiopia owing to the large population of 30.70 million sheep and 30.20 million goat heads (CSA, 2017). They account for 40% of cash income earned by farm households, 19% of the total value of subsistence food derived from all livestock production, and 25% of total domestic meat consumption (Adane & Girma, 2008). In addition, small ruminants have been significantly supporting the livelihood of smallholder farmers. They serve as living bank for their owners, cultural heritage of the communities, source of immediate cash need, medicinal value and insurance against crop failure especially where land productivity is low and unreliable due to erratic rainfall, severe erosion, frost, and water logging problems (Markos *et al.*, 2006; Solomon, 2009).

Despite the large numbers and the multiple roles small ruminants, farmer and national level small ruminant productivity in Ethiopia, is considered low as compared to productivity levels in developed livestock industries of the world (Solomon *et al.*, 2010). This low level of productivity could be attributed to several factors, chief among them is low genetic potential of the animals (Girma *et al.*, 2010). However, successful and sustained genetic improvement programs of indigenous breeds and populations of small ruminants under smallholder production condition are rare (Kosgey *et al.*, 2006; Markos *et al.*, 2006).

To satisfy the growing demand for animal products, under the national level sheep breeding program crossbreeding has been opted as an option using high producer exotic genotypes to improve the genetic potential of indigenous animals. Since 2007 G.C, large numbers of Dorper

sheep were imported from South Africa to form the backbone of crossbreeding program designed to utilize the fast growth rate and larger carcass of this animals with the native adaptability and toughness of local breeds.

In an attempt to support such endeavors International Center for Agricultural Research in the Dry Areas (ICARDA), International Livestock Research Institute (ILRI) and the Austrian University of Natural Resources and Applied Sciences (BOKU) in partnership with the National Agricultural Research System (NARS) in Ethiopia designed and implemented a new approach called community based breeding programs (CBBP) for four selected sheep breeds (Menz, Bonga, Horro, and Afar) (Aynalem *et al.*, 2011).

Since 2012 G.C, Debre Birhan Agricultural Research Center (DBARC) crossbreeding has been implemented at villages in the lowland areas of North Shewa and around Debre Birhan town using pure and 50% blood level Dorper rams to improve the genetic potential of local sheep. In addition, the research center with the collaboration of ICARDA, ILRI and BOKU University has implemented CBBP in the Menz areas to improve the body weight and reproductive performances of Menz sheep through selection.

Village level genetic improvement through selection and crossbreeding programs are not effective since lambing in village flocks is thinly distributed across the year and usually not planned. This could expose most of the lambs to unfavorable seasons for both survival and growth. However, hormone-synchronized breeding could be a solution for achieving both planned and concentrated lambing. According to Solomon *et al.* (2016b) introducing hormonal oestrus synchronization in village genetic improvement program gives 160.5–190.1% more profit than selection under natural oestrous synchronization (Zelege *et al.*, 2015).

In addition coupling oestrous synchronization with artificial insemination (AI) hastens the dissemination of improved sheep genotypes. AI is well known for its use in animal breeding program to improve genetically and economically important traits through selection. AI is more efficient as determination of the stage of oestrus in ewes and enables sheep farmers to introduce superior genetic material, accelerates the rate and efficiency of genetic selection within a herd, maximizes the number of offspring from a desirable sire, and enables genetic exchange over wide geographical areas (Tsuma *et al.*, 2015).

To this end, to facilitate AI implementation in sheep CBBP areas, ICARDA supported practical oriented trainings were given for experts (researcher) coming from different agricultural research centers. In addition, aiming to achieve planned and concentrated lambing efforts were made by Livestock and Irrigation Value chains for Ethiopian Smallholders (LIVES) project on sheep estrus synchronization in Menz and selected sheep breeds in Ethiopia (Solomon *et al.*, 2016b; Zeleke *et al.*, 2015).

On the other hand large number of high producer exotic sheep breeds (both male and female) were bought with high price and imported to Ethiopia for crossbreeding programs. However, by maintaining minimum effective population size (N_e) AI could be a solution for import fewer exotic genotype. In addition, AI allows to produce large number of crossbreed lambs from imported exotic sheep within few years this enable to accelerate the rate of genetic improvement programs.

In the CBBP large numbers of rams were selected for breeding purpose. This attributes decreasing selection intensity and hence slower genetic progress. Thus AI enables sheep

farmers in the CBBP to accelerate the rate of genetic progress by maximize the number of offspring from a desirable fewer best rams.

Evidences showed that hormonal synchronization with collaboration of AI enabled to achieve higher selection intensity and hence faster genetic progress. Though there are few efforts on hormones estrus synchronization in the local sheep, instead of AI, rams were used for mating. In general, small ruminant AI as a genetic improvement tools is not yet well implemented in indigenous sheep in Ethiopia. Therefore, the objective of this study was

General objective

- ✓ To evaluate estrus synchronization and AI in Menz sheep

Specific objective

- ✓ To asses Menz sheep responses to various estrus synchronization protocols
- ✓ To evaluate the success of AI in ewes inseminated with Dorper and Menz semen

2. LITERATURE REVIEW

2.1. Characteristics of Menz Sheep

The Menz breed belongs to the indigenous short fat tailed sheep population (Wollo, Farta, Tikur, Simien and Sekota). They are predominantly found in North Showa zone of Amhara regional state. They are also called Legegora, Shewa, Abyssinian and Ethiopian highland sheep (Solomon, 2008). Menz sheep breed is adapted to the very cold climate of the sub-alpine (2500 - 3200 m.a.s.l.) and are tolerant to drought and endo-parasite infection. This breed has no particular distinguishing colour. However, plain red, white and black coat colours are the dominant colours observed in Menz sheep. 99.1% of the Menz ewes are polled whereas 92.3% of the rams are horned (Tesfaye, 2008).

Menz sheep are kept primarily to generate income followed by meat, manure, coarse wool and as means of saving. Menz rams attains sexual maturity at average age of 10.5 months. Whereas age at first lambing, lambing interval, twining rate and lifetime productivity of Menz ewes are 470.1 days, 255.1 days, 1% and 9.3 lambs respectively. Body weight of mature (having 2 and above pairs of permanent incisor) Menz ram and ewes are 24.9 ± 0.67 kg and 22.3 ± 0.13 kg, respectively (Rekik *et al.*, 2016; Tesfaye, 2008).



Figure 1: A mature Menz ram (left) and ewe (right): Source (Tesfaye, 2008)

2.2. Community Based Breeding Programs

Since the early 1960s G.C, several efforts have been made to improve genetic potential of indigenous sheep population through cross breeding using high producer exotic genotypes (Markos, 2006). However, these genetic improvement programs produced no significant effects on sheep productivity (Solomon *et al.*, 2013). The major shortcoming of this project is that they do not address fully the farmers' preferences under low-input systems and also fail to consider the indigenous practices (Solomon *et al.* 2011, 2013).

Failure of the conventional breeding schemes has led to community-based breeding schemes being suggested as viable options for the genetic improvement programs of small ruminants in low-input, smallholder production systems (Solomon *et al.*, 2013). The project was initiated jointly by ICARDA, ILRI, BOKU University, and NARS in Ethiopia in four selected sheep breeds (Menz, Bonga, Horro, and Afar) (Aynalem *et al.*, 2011). Unlike the conventional top-down approach, CBBP involve the local community at every stage from planning to operation of the breeding program (Solomon *et al.*, 2013). Promising achievements of CBBP in productivity improvement of indigenous sheep have been reported. These include significant improvement of annual income of smallholder farmers from sale of sheep, and significant improvement of live body weight, body conformation and appearance of sheep (Zelalem *et al.*, 2015).

2.3. Oestrus (Ovarian) Cycle of Sheep

The average estrous cycle (time from one ovulation to the next) is typically 17 days in the ewe. Proestrus, estrus, metestrus, and diestrus are the phase of estrus cycle in sheep. The period of time when the ewe is receptive to the ram and will stand for mating is called estrus.

Duration of estrus is lasts approximately 24 to 36 hours. Ovulation occurs in mid to late-estrus (~24 hours after the onset). Metestrus begins with the cessation of estrus and lasts for about 3 days. It is the period of the formation of corpus luteum (CL). Diestrus is the period of the estrus cycle when the CL is fully functional. It usually extends from day 4 to day 13-15 of the cycle. Proestrus begins with the regression of the CL and drop in progesterone and extends to the start of estrus. Rapid follicular growth is occurring during this period (Schoenian, 2012; Pawel *et al.*, 2011).

2.3.1. Hormonal control of the estrus cycle of Sheep

Estrus cycle is controlled by GnRH (Gonadotrophic Releasing Hormone) released by the hypothalamus. Just before the onset of estrus, the pituitary gland under the control of the hypothalamus in the brain releases an increasing amount of Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH). LH stimulates the final maturation of the follicle containing the eggs (oocytes) and stimulates the follicle to produce the hormone estrogen. Estrogen brings the ewe into behavioral estrus or 'heat' (Girma, 2008; Kennedy, 2012).

After ovulation, under the influence of LH the cells inside the ruptured follicle begin to grow and undergo changes to form the CL. The progesterone is called the hormone of pregnancy and produced from CL. The CL is destroyed if fertilization is does not occur. Prostaglandin F2alpha (PGF2 α) hormone produced by the inner wall of the uterus destroy CL. The reduction in progesterone concentration is consequently followed by a rise in gonadotropin release (FSH and LH), development of another follicle(s) and egg(s), and recurrence of heat. This cycle will continue throughout the reproductive life of the ewes and will only be interrupted by pregnancy, disease, under or overfeeding (Kennedy, 2012; Tsuma *et al.*, 2015).

2.4. Estrus Synchronization

Estrous synchronization is the manipulation of the estrous cycle of ewes to come to heat within a predefined time frame (36 to 96 hours). In small ruminants, estrous synchronization is achieved either by injection of PGF2 α by reducing the length of the luteal phase of the estrous cycle or by extending the cycle artificially with application of exogenous progesterone or more potent progestogens (Girma, 2008).

Oestrus synchronization enhances the efficiency and cost of artificial insemination (AI) as many synchronized ewes can be inseminated at the same time by the same AI technician. Oestrus synchronization, especially under intensive sheep production systems, can utilize protocols that enable fixed time AI following a synchronization treatment irrespective of whether the ewes are seen on heat or not (Tsuma *et al.*, 2015).

2.4.1. Hormones used for estrous synchronization

Progesterone

The progesterone is called the hormone of pregnancy and produced by the CL of the ovary following ovulation. Its task is to prepare the uterus for accepting the fertilized egg, and to maintain pregnancy by preventing recurrence of the oestrus cycle during gestation. Progestins are the synthetic compound that has the properties of progesterone and mimic the function of the CL. Progestogens can be provided by feeding Melengestrol acetate (MGA), implants under the skin (Synchro-Mate B®), sponges and controlled internal drug release (CIDR) inserted into the vagina (Abecia *et al.*, 2012; Schoenian, 2012).

Intra-vaginal sponges have been the traditional method of synchronizing estrus in ewes. They contain progestogens that are effective at lower doses than natural progesterone. The most commonly used commercial available sponges are fluorogestone acetate (FGA) 20 mg/sponge,

marketed as Chronogest, and medroxyprogesterone acetate (MAP) 60 mg/sponge, marketed as Veramix widely used with pregnant mare's serum gonadotropin (PMSG). Intra-vaginal sponges have high retention rates (>90%) and females usually show estrus 24 to 48 hours after removal (Rekik *et al.*, 2016; Schoenian, 2012; Wildeus, 2014).

The CIDR and sponge are inserted in the ewe's vagina for 8 to 17 days following the injection of PMSG. As long as the CIDR and sponge is in place it releases progesterone into the bloodstream of the ewe, and consequently blocks secretion of FSH and LH from the anterior pituitary gland and as a result follicles do not develop and grow on the ovary, accordingly blocking the estrous cycle. When the CIDR and sponge are removed, there is rapid fall in progesterone level to the normal cycle and the block it had on FSH and LH secretion is removed, results secretion of FSH and LH, follicular development and maturation in the ovary, and oestrous can be expected in 24 to 36 hours of removal (Abecia *et al.*, 2012; Tsuma *et al.*, 2015).

Prostaglandin F2alpha

PGF2 α is a naturally occurring luteolytic hormone that has been utilized to synchronize estrus and induce abortion in ruminants through induction of CL regression. In the absence of an embryo, uterine concentration of PGF2 α increase during the late luteal phase (Shaikh *et al.*, 2015).

After ovulation the CL develops from residual follicular granulosa and thecal cell. Based on morphological and biochemical criteria and on the follicular source of origin, developed CL is composed of at least two steroidogenic cell types. Small luteal cells appear to be of thecal cell origin and respond to LH with increased secretion of progesterone. Large luteal cells are of

granulosa cell origin and contain receptors for PGF2 α and appear to mediate the luteolytic actions of this hormone (Niswender *et al.*, 2000).

If pregnancy does not occur, the CL must regress to allow follicular growth and ovulation. Luteal regression is initiated by PGF2 α of uterine origin in ruminant. Luteal cells are known to have PGF2 α receptors on the plasma membrane and direct inhibitory effects of PGF2 α on luteal progesterone secretion. During the early luteal phase, administration of PGF2 α to domestic ruminants does not induce luteolysis. Prostaglandin-based protocols are only effective only when an active CL exists at the time of application on the ovary and are restricted to use during the breeding season (Niswender *et al.*, 2000; Schoenian, 2012).

PGF2 α (Lutalyse™; Pharmacia & Upjohn) and the prostaglandin analogue cloprostenol (Estrumate®; Bayer, Shawnee Mission, KS) are the two commonly used prostaglandins. Prostaglandin-based estrous synchronization systems control the estrous cycle by terminating the luteal phase through regression of the CL. The regression of CL via prostaglandin, causing removal of the negative block progesterone has on FSH and LH release. When this progesterone block is removed, follicles develop, mature and the ewes come on heat within 36 to 72 hours of prostaglandin injection (Schoenian, 2012; Tsuma *et al.*, 2015).

When a single injection of prostaglandin is given to a flock of cycling ewes, 60 to 70 percent of the flock will exhibit a synchronized estrous beginning 30 to 48 hours later (Solomon *et al.*, 2016b). Because not all stages of the estrous cycle are similarly receptive to treatment, a double injection system 7 days or 11 days apart, to make sure all functional CL regress is the most widely used approach in small ruminant (Schoenian, 2012; Tsuma *et al.*, 2015).

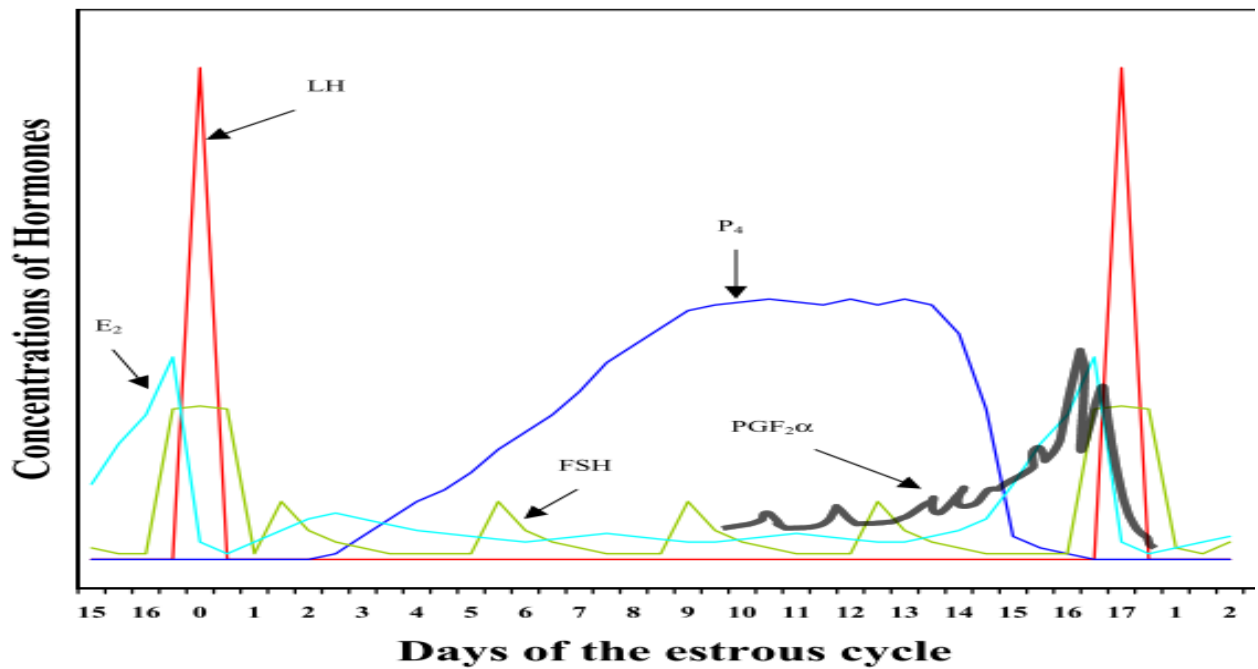


Figure 2: Estrus cycle in sheep

2.5. Artificial Insemination

To accelerate the rate of genetic gain and boost reproductive performance of livestock species various assisted reproductive technologies have been applied. AI is well known for its use in animal breeding program to improve genetically and economically important traits by selection of individuals (Morrell, 2011; Tsuma *et al.*, 2015).

2.5.1. Semen collection

Semen can be collected by artificial vagina (AV) or by electroejaculator. In most domestic animals semen is collected by means of an AV after allowing the male to mount on an oestrous female or a phantom (Morrell, 2011). The AV method is preferable because it does not stress the animal and results in the collection of higher quality semen. However, electroejaculator is less well tolerated by the male and it affects the semen quality and reduces the ability of spermatozoa to survive preservation (Bopape *et al.*, 2015; Faigl *et al.*, 2012).

2.5.2. Semen evaluation and processing

Collected semen is immediately evaluated for volume, appearance (i.e. color, contamination, etc.), concentration and motility, as well as later determination of sperm morphology and the presence of foreign cells. Once screened for normality, ejaculates used for AI are assessed for sperm concentration, morphology and sperm motility (Alm-packalén, 2009). Semen volume (varied from 0.3ml to 2ml) is measured with a calibrated collection grass. Normal color of ram semen is white to pale creamy (Steyn, 2003). The sperm cell concentration (normal: 3.5 to 6 billion) can be examined using spectrophotometer, and with a good phase contrast microscope mass motility (scoring system from 0 – 5), individual progressive forward movement (normal: 80-90%) and morphology (normal: 70-80%) can be evaluated (Faigl *et al.*, 2012). Depend on individual rams, testicle size, age of the ram, frequency of collection, feeding, body condition and health of the ram semen volume, motility and concentration are varied (Steyn, 2003).

The qualified semen to be used for AI is then processed and safely stored. The processing includes dilution of the ejaculate with a semen extender in order to provide an insemination dose and to nourish the spermatozoa and to provide a good environment for semen survival. When semen is for frozen use, glycerol is added in the diluent as a cryoprotectant to protect the spermatozoa from being destroyed during the freezing process. The extended semen is then packed in labeled straws and stored frozen in liquid nitrogen in tanks at -196°C (Steyn, 2003; Tsuma *et al.*, 2015).

2.5.3. Insemination techniques

Vaginal insemination

Vaginal is the simplest form of insemination and involves depositing fresh semen into the anterior vagina without any attempt to locate the cervix. The procedure is very fast, easy to

perform under field conditions and may result in low but still acceptable pregnancy rates with fresh or chilled semen. However the pregnancy rates obtained with frozen semen are not acceptable (Faigl *et al.*, 2012; Schoenian, 2012).

Cervical insemination

Cervical insemination is the preferred option in small ruminants and relatively easy method of insemination techniques. The cervix is located, via speculum with a bit of lubrication fitted with a light source and inserted into the vagina to a depth of 10-13 cm. The semen is deposited into the first fold of the cervix. Conception rates with fresh or chilled semen are good (65 to 75%), but generally unacceptably low (10 to 30%) with frozen thawed semen (Faigl *et al.*, 2012; Zeleke *et al.*, 2005).

Laparoscopic/intrauterine insemination

This is a specialized technique to be performed by a well-trained veterinarian and most effective. The reason to perform this technique is to utilize and to get good results with frozen semen and to inseminate more ewes per ejaculate with fresh semen: 20 million live sperm versus 120 million for cervical AI (Faigl *et al.*, 2012; Steyn, 2003). The semen is injected directly into the lumen of the uterus, and the same procedure is repeated on the other uterine horn. The procedure takes 2 to 5 minutes per ewe. The main disadvantages are the need for expensive laparoscopic equipment, invasive surgery (animal welfare issues), and the technical expertise needed to perform the procedure (Faigl *et al.*, 2012; Schoenian, 2012).

2.6. Main Factors Affecting Success of AI Using Fresh Semen in Sheep

2.6.1. Female associated factors

Breed

The ewe breed has been found to have a significant source of variation in fertility after AI (Fukui *et al.*, 2007; Karagiannidis *et al.*, 2001). The variation may be due to differences in the morphometric characteristics of the cervix and in the meantime of ovulation and ovulation rate between breed of ewes (Kaabi *et al.*, 2006).

Age

Young and maiden ewes have lower fertility than adult ewes, this probably due to impaired sperm transport combined with low mucus production in the cervical canals during oestrus. The best fertility rates in ewes recorded aged between 1.5 and 4.5 years; beyond this age, fertility declined remarkably (Anel *et al.*, 2005). Santolaria *et al.* (2011) conclude that ewes for insemination groups should be 2 to 5 year old while younger and older ewes should be used for natural mating.

Body condition score

Body condition score (BCS) has proved valuable as a management tool for subjectively assessing the nutritional status of ewes. Body condition directly affects hypothalamic activity and GnRH secretion. Good nutrient intakes respond most rapidly to the onset of the breeding season and continue to respond with an increase in ovulation rate (Maksimovic, 2012). Fukui *et al.* (2010) conclude that regardless of the body weight, body nutritional condition is an important factor next to ewe age, influencing the fertility of Suffolk ewes after AI.

Lambing-AI interval

To allow uterine involution resting period for the ewe after lambing is crucial. However, sometimes the increasing reproductive rate imposed by the demanding production system

involves short resting periods from lambing to AI, which affects fertility in a negative way. Anel *et al.* (2005) conclude that AI fertility decreased when the lambing-insemination interval was lower than 10 weeks.

Season

Seasonal effect was more important in cervical than laparoscopic insemination. In cervical insemination, semen is deposited in the external portion of the cervix and the sperm transport is affected by cervical mucus quality. Photoperiod could alter cervical mucus making it scarcer and more viscous. In result sperm transport in the cervix can be restricted with (Anel *et al.*, 2005).

2.6.2. AI associated techniques

Semen collection frequency

Semen volume and sperm concentration is declines progressively with increase in the ejaculation frequency. Maximum proportion of viable cells was obtained in the second ejaculate. The use of the second and/or a mixture of second and third ejaculates would improve the results in AI (Yotov *et al.*, 2011). The general recommendation to improve semen quality for AI is establish a routine of semen collection two-three times per week (two collections per day/per ram) on different and non-consecutive days (Santolaria *et al.*, 2011).

Time of insemination

The ewe breeds has different meantime of ovulation and ovulation rate (Salamon & Maxwell, 1995). The best time of insemination for Chios and Chios x Vlachiki breeds are 48 and 72 h after sponge withdrawal, while for Vlachiki breed a better conception rate is obtained when fixed insemination is applied 48 and 60 h after sponge withdrawal (Karagiannidis *et al.*, 2001).

Semen deposition site

Vaginal insemination is the simplest form of insemination, which could result in the lowest conception rate. Cervical and trans-cervical methods of insemination is relatively easy method of insemination, could give much higher pregnancy rate, however, laparoscopic insemination is the most effective method. But this method is the most expensive and complicated method of insemination (Santolaria *et al.*, 2011; Schoenian, 2012).

2.6.3. Synchronization Protocols (Progesterone vs. prostaglandin)

Progesterone and PGF2 α are the primary hormones used for oestrous synchronization in ewes. Progesterone based protocols extend the length of the luteal phase artificially with application of exogenous progesterone while PGF2 α based protocols reduce the length of the luteal phase of the estrous cycle by regression of the CL (Girma, 2008; Tsuma *et al.*, 2015).

Prostaglandin-based synchronizations protocols are not applicable during seasonal anestrus, whereas exogenous progesterone in combination with gonadotropin can be used to induce and synchronizes estrus in anovular ewes (Wildeus, 2014). Prostaglandin-based protocols are only effective only when an active CL exists at the time of application on the ovary and are restricted to use during the breeding season (Niswender *et al.*, 2000; Schoenian, 2012).

Prostaglandin-based protocols have advantage over progesterone methods, it is relatively cheaper, yield a “clean” oestrous with no residues in the vaginal tract and easily administered by intramuscular injection thus animal management and welfare are improved compared to intravaginal devices (Omontese *et al.*, 2016). Previous study Gatti & Ungerfeld (2012) showed that sexual attractiveness of ewes synchronized with prostaglandin based protocols were higher compared to ewes synchronized with intravaginal sponges (MAP).

3. MATERIALS AND METHODS

The study had on-station and on-farm experiments. The on-station experiment were conducted at DBARC and the on-farm experiment were conducted at Menz area (Molale, Sinamba and Dargegn villages) where community based sheep genetic improvement program were implemented.

3.1. Study Area

DBARC is located 120 km North-east of Addis Ababa at an altitude of 2,765 m.a.s.l. and at a longitude of 39°39'10"E and latitude of 09°36'23"N. Debre Birhan is characterized by a bi-modal rainfall pattern, where the main rainy season is from June–September accounting for 75% of the total rainfall, an erratic unreliable short rainy season is February/March–April/May and a dry season October–January. Based on the meteorological data obtained from DBARC, the average annual rainfall is 923 mm. The mean annual maximum temperature is 19.75°C between June and August, while the mean annual minimum temperature is 6.86°C in November. The center has about 268 hectare land most of which is allocated for grazing.

Menz area is located in North Shewa administrative zone of Amhara regional state. There are five districts in Menz those are Menz Mama, Menz Gera, Menz Lalo, Menz Keya and Gishe. Menz Mama and Menz Gera are the two districts where community based sheep genetic improvement program were implemented. The Menz area is characterized by a low-input sheep-barley production system with a bi-modal rainfall pattern, where the main rainy season is from June to September and an erratic unreliable short rainy season is expected in February and March (Tesfaye *et al.*, 2010). Based on the meteorological data obtained from DBARC the

mean annual maximum temperature is 18.63°C between June and August, while the mean annual minimum temperature is 7.26°C in November.

The community based sheep genetic improvement program sites were Molale village which is located in Menz Mama District whereas Sinamba and Dargegn villages which are located in Menz Gera district (Figure 1). Menz Mama located 222 km north east of Addis Ababa at an altitude of 3047 m.a.s.l. and at a longitude of 39°39'41"E and latitude of 10°07'17"N. The annual rainfall is 1403 mm. Menz Gera located 280 km North east of Addis Ababa at an altitude of 3084 m.a.s.l. and at a longitude of 39°39'37"E and latitude 10°18'52"N. The annual rainfall is 938 mm.

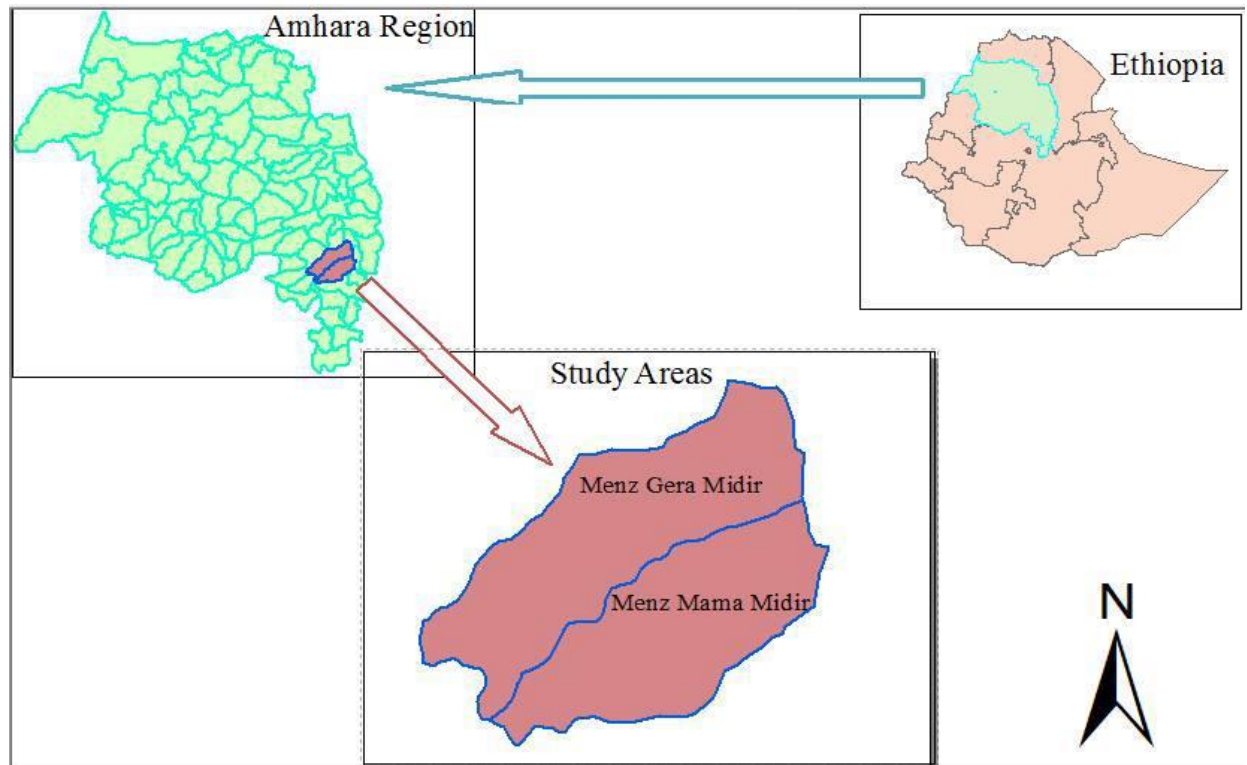


Figure 3: Approximate locations of the CBBP sites: Menz Gera (Mehal-Meda) and Menz Mama (Molale): Source (Kahsa, 2014)

3.2. Experimental Animals and Their Management

The ewes were scored for body condition based on (Girma and Alemu, 2008) during oestrous synchronization and scored 1 to 4.

On station experiments

A total of 184 sheep (89 Menz ewes and 4 Menz rams, 87 Menz ewes and 4 Dorper rams) were selected for pure and cross breeding AI experiment respectively. Rams age of 5-7 years and their BCS are 3.0 and above, and ewe's parity ranges from 1 to 5 and not pregnant, which their BCS are 2.5 and above, and ewes not suckling were used in this trial.

All experimental ewes were housed in the night and allowed to graze during the day on natural pasture daily for 6-7 hrs. Rams selected for semen collection were housed in the night and the day for about 15 days before semen collection was started. Rams were trained to serve AV one times per day in non-consecutive days for a week. All experimental animals were fed quality hay ad libitum. The dominant natural vegetation is *Andropogon longipes* grass with variable proportions of *Pennisetum*, *phalaris* and *Festuca* species and also the legume *Trifolium* spp. In addition, the experimental animals received mixed commercial concentrate based on their body weight 200, 300 and 500 g head/day consisting of 33% noug (*Guizotia abyssinica*) cake, 65.5% wheat bran, 1% limestone and 0.5% salt for Menz ewes, Menz and Dorper rams in the morning and evening respectively. The mixed commercial concentrate feed had 19.9% crude protein, 79.3% total digestive nutrient. The animals had free access to fresh water twice a day. The house was provided with necessary arrangement for feeding, watering, draining and were well ventilated. As a routine flock health management practice of the research center the experimental animals were drenched against internal parasites (Rafoxanide at 1ml/4kg body weight, Chanelle pharmaceuticals manufacturing Ltd., Ireland and Tetraclozash-900® at 1

bolus/30kg body weight, Ashish life Science Pvt. Ltd., India) in August 2017 and were vaccinated against Ovine Pasteurellosis, Peste des Petitis Ruminants (PPR), Sheep and Goat Pox, Blackleg and Anthrax (National veterinary institute, Debrezeit, Ethiopia) in the different seasons of the year before the beginning of the experiment. Moreover, the experimental animals were sprayed against ectoparasites (Diazinol 60% E.C at 1ml/1Lit. of clean water, Kafrel Zayat pesticides and chemicals CO., Egypt) in July 2017.

On farm experiment

In October 2017, a total of 101 animals (89 Menz ewes and 12 Menz rams) were selected for on-farm Menz sheep pure breed AI experiment. Experimental animals were selected on the bases of the following criteria. Ram's age ranged from 3 to 5 years and their BCS are 3.0 and above, ewe's parity ranges from 1 to 5 and that gave at least one birth and not pregnant, with BCS of 2 and above, and not suckling were selected from households (HHs) participated in the CBBP. Non pregnant ewes were identified using ultrasonography (B-Ultrasonic Diagnostic 23500/1000 Minitub GmbH). Rams were trained to serve AV one times per day in non-consecutive days for a week. Semen was collected from rams which were selected to give natural service in the CBBP. Those rams were selected from HHs participated in CBBP based on their breeding values and other important parameters including body conformation, color, horn size and orientation, testicle size, and libido.

Before the commencement of the actual experiment, the experimental animals were isolated from three villages (Sinamba, Dargegn and Molale) of individual HHs flock participated in the sheep CBBP. The experimental animals were maintained in a group for about five weeks and were housed in the night and allowed to graze during the day on grazing pasture on their respective villages in order to flush the animals. Rams selected for semen collection were

housed in the night and the day, and were feed hay that was prepared from natural pasture, faba bean and lentil straw as ad libitum for about 10 days before semen collection was started. The experimental animals were drenched against internal parasites (Albendazole 300 mg at 1 bolus/30 kg body weight, Ashish life Science Pvt. Ltd., India), and were vaccinated against Ovine Pasteurellosis, Peste des Petitis Ruminants (PPR) and Sheep and Goat Pox (National Veterinary Institute, Debre Zeit, Ethiopia) in the different season of the year before the beginning of the experiment. After 10 days of insemination date the experimental animals were returned back to their respective HHs.

3.3. Protocols and Procedures

Animals were allocated into three experimental groups of 30, 28, and 31 for the on-station and 30, 30, and 29 ewes for the on-farm pure breeding experiment randomly by blocking the animals for the parity, BCS and villages (for the on farm experiment only). For the on-station crossbreeding experiment animals were subdivided in to four experimental groups of 22, 24, 21 and 20 ewes randomly by blocking the animals for the parity and BCS.

Table 1: Schematics set up of protocols

Protocols	Prostaglandin-based		Intravaginal sponges		Time of AI (hrs)
	1 st injection	2 nd injection	Insertion	Withdrawal and PMSG injection	
Protocol 1	Day 0				48-51
Protocol 2	day 0	at day 7			48-51
Protocol 3	Day 0	at day 11			48-51
Protocol 4			Day 0	at day 12	48-51

Protocol one

All experimental ewes received single injection of 5 mg of the PGF2 α analogue dinoprost (1ml Enzaprost®; CEVA laboratories, Libourne, France) intramuscularly at unknown stage of estrous cycle.

Protocol two

Ewes were intramuscularly administered (Day 0) with 5 mg of the PGF2 α analogue dinoprost (1ml Enzaprost®; CEVA laboratories, Libourne, France) followed by another intramuscular injection of 1 ml Enzaprost® at day 7.

Protocol three:

Ewes were intramuscularly administered (Day 0) with 5 mg of the PGF2 α analogue dinoprost (1 ml Enzaprost®; CEVA laboratories, Libourne, France) followed by another intramuscular injection of 1 ml Enzaprost® at day 11.

Protocol four:

Animals were treated with intravaginal polyurethane sponges impregnated with 45 mg fluorogestone acetate (Syncro-part®; CEVA laboratories, Libourne, France). The intravaginal

progestogen sponges remained in situ for a period of 12 days in experimental ewes. All ewes were checked twice daily (morning and evening) to ensure that sponges remained in place during the treatment period. At sponge withdrawal, each ewe received an intramuscularly injection of 300 I.U. of equine chorionic gonadotropin (Syncro-part PMSG®; CEVA laboratories, Libourne, France).

In the on-station experiment two ewes in “single PGF2 α injection” group and one ewe in “double PGF2 α injection 11 days interval” group died due to unknown reason while one ewe in “double PGF2 α injection 11 days interval” group was discarded due to severe deterioration of their BCS. On the other hand in the on-farm experiment three ewes in “double PGF2 α injection 7 days interval” group and four ewes in “double PGF2 α injection 11 days interval” group were discarded because of the incidence of abortion which were caused by injection of early pregnant ewes with PGF2 α . One ewe in “double PGF2 α injection 7 days interval” and four ewes in “double PGF2 α injection 11 days interval” groups were discarded because of severe deterioration of their BCS. Data of these animals were not used during the analysis, and therefore, results are presented for 80, 78, 71 and 20 ewes for the “single PGF2 α injection”, “double PGF2 α injection 7 days interval”, “double PGF2 α injection 11 days interval” and progestogen protocols groups respectively.

3.4. Oestrus Observation and Artificial Insemination

Ewes were housed during oestrus identification and were observed for oestrus starting from 24 to 48 hrs post hormone administration on the exercise yard of the barn. Ewes at heat were identified using proven ram fitted with an apron. Standing to be mounted was the key sign used to determine oestrus response. Semen was collected from rams trained to serve an AV,

after allowing the rams to mount in estrous ewes. The AV was prepared from lubricated liner which was inserted into an outer jacket and the liner was folded back and secured over the end of the outer jacket. The space between the liner and the outer jacket was filled with 50 to 55°C water to correct the internal AV temperature to had 42 to 45°C. The desired pressure stimulated to the erect penis of the rams to cause ejaculation was obtained by adding the air in to the space through the tap. The ejaculated semen was deposited into sterile calibrated semen collection glass attached to one end of the liner (Morrel, 2011). Semen was collected from rams 2-3 times daily on non-consecutive days.

Collected semen was immediately evaluated for volume, appearance (color, contamination), motility and concentration. The volume of semen per ejaculation was measured with a calibrated collection glass and the color of the semen was scored subjectively by looking and classified into, milky, watery, thin creamy, creamy and thick creamy (Evans and Maxwell, 1987). Then the semen was immediately stored at 37.5°C in a water bath to evaluate the fresh semen for mass motility and sperm concentration.

The sperm cell concentration was evaluated using AccuRead IMV Technologies SA, 232 Spectrophotometer (Evans and Maxwell, 1987). Sperm cell concentration was estimated using micropipette to take normal saline (Na 9gm) and put 4 ml of normal saline on the UV Macro cell (UV Macro Cell 2.5 ml - 4.5 ml, Great Britain) and using micropipette take 10 micro litter of fresh semen and put on the 4 ml of normal saline and mix gently and measure the concentration using AccuRead IMV Technologies SA, 232 Spectrophotometer. Sperm mass motility was estimated subjectively by using phase contrast microscope (Scope Technology Scope Photo 3.0.12.). For that semen was taken with pipetter, dropped on the slide and covered with cover slip and observed with the 10x magnification on the objective lens under

microscope. The mass motility was graded zero to five (0-5) score based on the passion of the wave motion as described by (Evans and Maxwell, 1987). According to these methods:

5. Very good (dense, cloudy and very rapidly moving waves 90% or more of the spermatozoa were active).
4. Good (dense, vigorous wave movement 75-90% of sperm cells are active).
3. Fair (small, slow moving waves 40-70% of sperm cells are active).
2. Poor (some movement of sperm is visible. 20-40% of sperm cells are alive but with poor motility
1. Very poor (only about 10% of spermatozoa active (weak movement around).
0. Dead (all sperm cells are motionless).

Fresh semen was diluted with OviXcell (preservation medium for ovine semen, IMV Technologies, France) containing antibiotics to provide insemination does of 150 million spermatozoa. The extender was gently shaken and warmed up to 37.5°C using a water bath before semen dilution. The extended semen was then packed in 0.25 ml straws (IMV technologies product, France) manually and was labeled with information regarding the rams from which it was collected (sire code, ejaculation number and breed). Fixed time cervical AI was performed in estrous ewes 48-51 hr following Enzaprost® and PMSG injection with 0.25 ml diluted fresh semen (approximately 150×10^6 sperm/ml and its mass motility score 3 and above) using a speculum equipped with a light source and an insemination gun, the semen was slowly released into the cervix.

3.5. Statistical Analysis

The effect of synchronization protocols, BCS, parity and management on the oestrous response of ewes was analyzed using a chi-square test (SPSS version 20). The same statistical procedures were adopted to analyze the effect of synchronization protocols, genotype, BCS and parity on pregnancy, lambing and fecundity rates. The general linear model (GLM) procedures of SAS system (9.0) were used to run least squares analysis of variance to test for the effect of synchronization protocols, rams genotype, body condition, parity and sex of lambs on the litter size and litter weight.

The following model was used;

$$Y_{ijklmn} = \mu + P_i + G_j + B_k + Pr_l + S_m + \epsilon_{ijklmn}$$

Y_{ijklmn} = response variable

μ = the overall means

P_i = the fixed effect of i^{th} synchronization protocols

G_j = the fixed effect of j^{th} genotype

B_k = the fixed effect of k^{th} body condition

Pr_l = the fixed effect of l^{th} parity

S_m = the fixed effect of m^{th} sex

ϵ_{ijklmn} = the residual error

The effect of genotype and ejaculation frequency on ejaculation volume, mass motility and concentration of spermatozoa were analyzed using GLM procedures of SAS system (9.0).

The model is as follows;

$$Y_{ijk} = \mu + G_i + Ef_j + \epsilon_{ijk}$$

Y_{ijk} = Response variable

μ = the overall means

G_i = the fixed effect of i^{th} genotype (Doper and Menz)

E_{f_j} = the fixed effect of j^{th} ejaculation frequency

ϵ_{ijk} = the residual error

Pregnancy, lambing, fecundity rate and oestrous response were calculated as follows:

$$\text{Pregnancy rate} = \frac{\text{number of ewes pregnant}}{\text{number of ewes inseminated}} \times 100$$

$$\text{Lambing rate} = \frac{\text{number of lambs born alive}}{\text{number of ewes inseminated}} \times 100$$

$$\text{Fecundity rate} = \frac{\text{number of lambs born alive}}{\text{number of pregnant ewes}} \times 100$$

$$\text{Oestrous response} = \frac{\text{ewes show oestrus}}{\text{ewes administered hormone}} \times 100$$

4. RESULTS

4.1. Lambing Distribution of Menz Sheep under Natural Oestrous

Lambing distribution of Menz sheep under natural oestrous over a year are presented in Table 2. The results indicated that though there were some peak lambing months, lambing under natural oestrous in Menz CBBP areas occurred throughout the year in scattered manner. The results showed that 46.4% of lambing in the study area occurred during unfavorable seasons of the year (January to June). The peak lambing months accounted 31.7% of the total lambing. The peak lambing months of Menz sheep were December (10.9%), September (10.5%) and January (10.3%). Whereas the lowest lambing was observed in April (5.4%) and March (6.1%).

Table 2: Lambing distribution of Menz sheep over a year in the CBBP, Menz areas, from 2010 to 2017 G.C

Factor	Lambing month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Lambs born (n)	1294	1186	760	676	943	961	1062	1009	1316	985	993	1368
Lambing %	10.3	9.4	6.1	5.4	7.5	7.7	8.5	8.0	10.5	7.8	7.9	10.9

Lambing % = Lambs born each months divided by total lambing in a year

4.2. Oestrous Response of Menz Sheep Synchronized with Different Protocols

Effect of synchronization protocols, BCS, parity and management on oestrus response of Menz sheep are presented in Table 3. In the present experiment, out of the 249 ewes treated with different oestrus synchronization protocols, 193 (77.5%) exhibited overt signs of oestrous within 48 hours of hormone administration. However, there was no significant difference in percentage of ewes exhibiting overt signs of oestrus within 48 hours of hormone administration across protocols. Similarly, the results of double dose administration of PGF2 α at interval of seven and eleven days and single dose hormone administration haven't shown significant differences on oestrous response.

On the other hand the results indicated that ewes that had between 2.5 and 3.0 BCS had significantly higher ($p < 0.05$) response to hormonal treatment compared to ewes that had 2.0 BCS. Whereas difference in parity and management had no significant effect on the percentage of ewes exhibiting overt sign of oestrus within 48 hours.

Table 3: Effect of protocols, body condition, parity and management on oestrous response of Menz sheep

Parameters	No of Ewes synchronized	Oestrous response (%)
Protocols		ns
Single PGF2 α	80	73.8
Double PGF2 α 7 day interval	78	78.2
Double PGF2 α 11 day interval	71	77.5
Progestogens (FGA+PMSG)	20	90.0
Body condition		*
3.5 and above	32	71.9 ^{bc}
Between 2.5 and 3.0	197	80.7 ^{ab}
2.0	20	55.0 ^c
Parity		ns
Multiparous	114	74.6
Primiparous	135	80.0
Management		ns
On-station	172	78.5
On-farm	77	75.3
Overall	249	77.5

^{a, b, c} On the same column, numbers bearing the same superscript are not statistically different at $p < .05$; ns: not significant.

4.3. Success of Artificial Insemination in Menz Sheep

Effect of semen genotype and management on pregnancy, lambing and fecundity rates of Menz ewes are presented in Table 4. The overall pregnancy, lambing and fecundity rates recorded following oestrous synchronization and artificial insemination was 59.1, 58.0 and 98.2% respectively. There was a significant difference in pregnancy and lambing rates among the genotypes. Accordingly ewes inseminated with Dorper semen had higher ($p < 0.05$) pregnancy and lambing rates as compared to ewes inseminated with Menz. No significant differences were observed due to management differences among ewes inseminated with Menz semen in pregnancy, lambing and fecundity rates.

Table 4: Effect of genotype and management on pregnancy, lambing and fecundity rate in Menz sheep

Experiment	Management	No Ewes inseminated	Pregnancy rate (%)	Lambing rate (%)	Fecundity (%)
			*	*	ns
Pure Breeding	On_station	67	55.2 ^a	56.7 ^a	102.7
Pure Breeding	On_Farm	58	46.6 ^a	43.1 ^a	92.6
Cross Breeding	On_station	68	73.5 ^b	72.1 ^b	98.0
Overall		193	59.1	58.0	98.2

^{a, b} On the same column, numbers bearing the same superscript are not statistically different at $p < 0.05$; ns: not significant.

4.4. Pregnancy, Lambing and Fecundity Rates in Menz Sheep

Effect of synchronization protocols, BCS and parity on pregnancy, lambing and fecundity rates of Menz sheep are presented in Table 5. The present findings revealed that variation in synchronization protocols between ewes didn't bring significant difference on pregnancy; lambing and fecundity rates. Moreover, Parity and BCS did not significantly affect pregnancy, lambing and fecundity rates in Menz sheep.

Table 5: Effect of protocols, body condition and parity on pregnancy, lambing and fecundity rate in Menz sheep

Factor	No of Ewes inseminated	Pregnancy rate (%)	Lambing rate (%)	Fecundity (%)
Protocol type		ns	ns	ns
Single PGF2 α	59	50.8)	49.2	96.7
Double PGF2 α 7 day interval	61	59.0	57.4	97.2
Double PGF2 α 11 day interval	55	61.8	61.8	100.0
Progestogens (FGA+PMSG)	18	77.8	77.8	100.0
Body condition		ns	ns	ns
3.5 and above	23	39.1	34.8	88.9
Between 2.5 and 3.0	159	62.3	61.6	99.0
2.0	11	54.5	54.5	100.0
Parity		ns	ns	ns
Multiparous	85	57.6	56.5	98.0
Primiparous	108	60.2	59.3	98.5

4.5. Litter Size and Litter Weight in Menz Sheep

Effects of protocols, genotype, BCS, parity and sex on litter size and litter weight are presented in Table 6. No significant differences in litter size and litter weight across protocols were observed. However, there was significant difference in litter weight among the genotypes. Dorper x Menz lambs had significantly ($p < 0.05$) heavier litter weight compared to pure Menz lambs. The present findings showed that BCS, parity and sex of the lamb did not significantly affect litter size and litter weight after estrous synchronization and artificial insemination in Menz ewes.

Table 6: Effect of protocol, genotype, body condition, parity and lamb sex on litter size and litter weight in Menz sheep

Parameters	n	Litter size (LSM \pm SE)	Litter weight (LSM \pm SE)
Protocols		ns	ns
Single PGF2 α	29	1.0	2.9
Double PGF2 α 7 day interval	34	1.03	3.0
Double PGF2 α 11 day interval	31	1.09	3.0
Progestogens (FGA+PMSG)	14	1.0	3.1
Genotype		ns	*
Menz pure	61	1.03	2.8 ^a
Dorper x Menz	47	1.04	3.3 ^b
Body condition		ns	ns
3.5 and above	8	1.0	2.6
Between 2.5 and 3.0	94	1.04	3.1
2.0	6	1.0	2.6
Parity		ns	ns
Multiparous	46	1.04	3.0
Primiparous	62	1.03	3.0
Sex		ns	ns
Male	52	1.02	2.9
Female	56	1.05	3.1
Overall	108	1.04	3.0

^{a, b, c} On the same column, numbers bearing the same superscript are not statistically different

at $p < .05$; ns: not significant.

4.6. Effect of Genotype and Ejaculation Frequency on Semen Quality Parameters

Least square means for the effect of genotype and ejaculation frequency on the ejaculation volume, mass motility and spermatozoa concentration are presented in Table 7. There was difference in ejaculation volume and concentration of spermatozoa among the genotypes ($P<0.05$). The results showed that ejaculation volume and concentration of spermatozoa were higher ($P<0.05$) in Dorper rams compared to Menz. Ejaculation frequencies had significant ($P<0.05$) effect on ejaculation volume. Thus semen volume was higher ($P<0.05$) in first ejaculate compared to second and third ejaculates respectively. However, the volume of the second and third ejaculates didn't show significant difference. Moreover, no significant differences in mass motility and concentration across ejaculation frequencies were observed.

Table 7: Semen quality parameters (LSM \pm SE) of Menz and Dorper Breeds

Factors	n	Parameters		
		Volume	Mass-motility	Concentration
Genotype		**	ns	**
Menz	125	0.62 ^a	4.2	4.11 ^a
Dorper	68	0.92 ^b	4.1	6.04 ^b
Ejaculation number		*	ns	ns
1 st Ejaculation	99	0.82 ^a	4.0	4.82
2 nd Ejaculation	53	0.61 ^b	4.3	4.66
3 rd Ejaculation	41	0.64 ^b	4.2	4.87
Overall	193	0.73	4.15	4.79

^{a, b} On the same column, numbers bearing the same superscript are not statistically different at $p < .05$; ns: not significant.

5. DISCUSSION

5.1. Lambing Distribution of Menz Sheep under Natural Oestrous

Natural lambing in Menz sheep occurred throughout the year in scattered manner (Table 2). In Menz CBBP areas, the peak lambing season accounted a maximum of only 31.7% of the total lambing per year commonly occurred following the main rainy season corresponding to availability of feed. The peak lambing months are September, December and January as most conception took place in April (short rainy season), July and August, which are the main rainy season in the Menz CBBP areas. It has been reported that lambing distribution of Menz sheep is year-round with a peak lambing in October and November reviewed by (Rekik *et al.*, 2015). The variation in the peak lambing months of Menz sheep could be attributed to variation in rainfall distribution corresponding to availability of feeds.

In Ethiopia, under smallholder small ruminant farming systems lambing is commonly distributed throughout the year. The results in Horro sheep in Oromia state and Tigray highland sheep in Tigray state indicate that natural lambing are distributed throughout the year (Solomon *et al.*, 2016b). The peak lambing months of Tigray highland sheep are January (25.2% of the lambing) and December (17.1% of the lambing) in Habes kebele and December (16.1% of the lambing) in Golgol-Nae'le kebele which is in consonance with peak lambing months of Menz sheep. The result from a study by Deribe, (2009) indicated that the lambing distribution of indigenous sheep in Alaba district in SNNP state is year-round with a peak lambing in May and November.

Though year-round distributed lambing could minimize risk of losing the whole lamb crop due to climatic change, could have a greater impact on survival and growth rates of the lambs born

during the unfavorable season of the year. Results in Table 2 showed that 46.4% of the lambing in Menz sheep occurred during dry season, which exposed most of the lambs to unfavorable seasons for survival and growth. Markos *et al.* (2006) found that Menz and Horro lambs born during the dry seasons had lower survival rate than those lambs born during the wet seasons. The results from a study by Solomon *et al.* (2016b) indicated that lamb mortality rates in Tigray highland sheep could reach as high as 80% for lambs born and raised in May-July.

Year-round lambing could also have a greater impact on flock genetic improvement programs. In Menz CBBP areas the maximum concentrated lambing accounted for only 10.9% of the lambs born in a year. This has a great implication on the effectiveness of village based small ruminant genetic improvement programs. Village based genetic improvement program through selection is commonly not effective since only few selection candidates would be available in each round of selection (Solomon *et al.*, 2016b).

Hormone-synchronized breeding of village flock could be a solution for achieving both planned and concentrating lambing. Lambing could be synchronized to match with low environmental burden (disease, cold shock, feed shortage) on the new born and the lactating dams.

5.2. Efficiency of Synchronization Protocols in Menz Sheep

Table 3 indicated the overall oestrus response with in 48 hours of hormone administration across the four synchronization protocols. The current work strongly revealed that oestrous could be effectively synchronized for achieving both planed and concentrated lambing in Menz sheep. The overall oestrus response attained in this trial was comparable to overall

oestrous response values (76.5%) reported by Solomon *et al.* (2016b) for four Ethiopian sheep population. Similarly Rekik *et al.* (2016) reported oestrous response of 79.3% in Menz ewes synchronized with two doses of PGF2 α injection administered eleven days apart. Findings of the current study were however, higher than those obtained by Zeleke *et al.* (2015) who reported 65% oestrous response in Menz sheep synchronized with single injection of PGF2 α , but lower than findings by Solomon *et al.* (2016b) who recorded oestrous response of 93.2% in Horro sheep synchronized with double PGF2 α injection nine days apart. The differences in oestrous responses reported in different studies might be due to differences in breed, season and overall management conditions of the animals.

While there were no significant difference across the four synchronization protocols in percentage of ewes exhibiting oestrus, ewes treated with progestogen-based protocol had higher response compared to ewes treated with PGF2 α based protocols. Studies by Derar *et al.* (2012); Rekik *et al.* (2016); Solomon *et al.* (2016b) indicated that progestogen-based protocols received ewes had significantly higher oestrous response rate compared to ewes subjected to PGF2 α based synchronization protocols. However, findings by Gonzalez-Bulnes *et al.* (2005); Naderipour *et al.* (2012); Omontese *et al.* (2014) have indicated that the efficiency of PGF2 α and progestogen-based protocols were not statistically significant in terms of oestrous response in ewes. The result from a study by Bitaraf *et al.* (2007) also showed that the efficiency of PGF2 α analogue and progestogen-based protocols were not varied in oestrous response in Nadooshani goats during the breeding season.

Since Menz sheep are expected to cycle throughout the year, the use of prostaglandin-based synchronization protocol advantages over weighs as compared to progestogen-based protocol.

Studies by Omontese *et al.* (2016); Rekik *et al.* (2016); Solomon *et al.* (2016b) indicated that prostaglandin-based protocols are relatively cheaper, easy to apply, rapidly metabolized with a minimum residue level and yield a “clean” oestrus with no residues in the vaginal tract. Previous study Gatti & Ungerfeld (2012) showed that sexual attractiveness of ewes synchronized with prostaglandin based protocols were higher compared to ewes synchronized with intravaginal sponges (MAP). However, in smallholder farmers under continuous mating system, if pregnant ewes were not identified precisely injection of early pregnant animals with prostaglandin will induces abortion.

Table 3 showed that oestrous responses of ewes were not varied across PGF2 α based protocols. In this study, no statistically significant advantage of two injection of PGF2 α at interval of seven and eleven days was observed over a single PGF2 α regime. In line with the present findings a study by Solomon *et al.* (2016b) indicated that differences in percentage of oestrous response following single or double injection of prostaglandin was not significant (oestrous response of 76% versus 96% respectively) for Ethiopian sheep population. However, Fierro *et al.* (2013) recommends that although various prostaglandin-based protocols can be used for estrus synchronization, a second prostaglandin injection improves estrus response when the stage of the estrous cycle at the first injection is unknown.

The estrus response recorded in this experiment under PGF2 α injection protocol was comparable to values reported by Rekik *et al.* (2016) and Solomon *et al.* (2016b) for Arsi-Bale sheep, Washera, Wollo and in Menz sheep. A similar comparable result was reported by Olivera-muzante *et al.* (2011) and Pope & Cárdenas (2004) in single and double PGF2 α regime. The percentage of ewes exhibiting oestrus in this trial was higher than previously reported by (Zelege *et al.* 2015; Solomon *et al.* 2016b) for Menz 65%, Awassi x Menz 55%

and Tigray highland sheep 57.5% respectively. However, estrus response attained in this study was lower than values reported by Solomon *et al.* (2016b) using two injection of PGF2 α for Horro sheep (oestrous response of 93.2%). Higher oestrus response (100%) was reported by Omontese *et al.* (2014) in Nigerian Ouda ewes.

The estrus response achieved in the current study under progestogen-based protocol was comparable to values reported by Jackson *et al.* (2014); Luther *et al.* (2007); Ungerfeld & Rubianes (2002). A similar finding was reported for goats from Spain by Lo'pez-Sebastian *et al.* (2007). Higher oestrous responses (100%) were reported by Dogan *et al.* (2004); Solomon *et al.* (2016b); Hashemi *et al.* (2006); Rekik *et al.* (2016); Omontese *et al.* (2014) in studies involving the use of different progestogen based protocols in different breed of ewes. However, estrus response attained in this study was higher than values reported by Boscos *et al.* (2002); Contreras-solis *et al.* (2009); Gonzalez-bulnes *et al.* (2005); Luther *et al.* (2007).

BCS has proved useful as a management tool for subjectively assessing the nutritional status of ewes. Ewes BSC was found to be a significant factor determining oestrous response to hormone treatment (Table 3). A similar result was also obtained by Solomon *et al.* (2016b) which indicated that ewes that had 2.5-3.0 BCS were better responsive to hormonal treatment than ewes that were in poor BCS. The Santoralia *et al.* (2011) review of factors affecting efficiency of synchronization indicated that high BCS has been associated with an increase of ovulation, with recommended BCS of 2.5–3.0 and a score of <2 resulting lowest pregnancy rates in sheep. Findings by Solomon *et al.* (2016a) revealed that body condition was determinant factor for the success of oestrous synchronization and AI in dairy cattle breeding program in Ethiopian.

Results pertaining the effects of parity on oestrous response were presented in Table 3. According to the present experiment oestrous response of ewes to hormone treatment was not significantly influenced by parity, which is in consonance with those of Luna-orocho *et al.* (2008) reported for goats. However, the result, contradict with findings of Solomon *et al.* (2016b); Ungerfeld and Sanchez-Davila (2012) who indicated that multiparous ewes were higher responsive to hormone treatment compared to primiparous ewes. Previous study by Véliz *et al.* (2009) show that oestrous response were statistically significant in multiparous and nulliparous does (oestrous response of 100% and 70% respectively). Anel *et al.* (2005) concluded that the best fertility rates in ewes were recorded between 1.5 and 4.5 years; and beyond this age, fertility declined remarkably.

In this study, oestrous response of ewes to hormonal treatment was not affected by management (Table 3). This may be due to manipulation of on-farm environment. Before the commencement of the actual experiment, ewes on the on-farm experiment were isolated from individual HHs flock and maintained in a group for about five weeks and allowed to graze in good grazing pasture in order to flush the animals.

5.3. Success of Artificial Insemination in Menz Sheep

The current pregnancy and lambing rates falls in an acceptable range, and is in agreement with those reported in the literature (Faigl *et al.*, 2012). According to Allaoui *et al.* (2014) in sheep, fertility rates ranges from 60 and 100% qualified as acceptable performance, in this regard the present study revealed that implementation of fresh semen AI in CBBP areas as a genetic improvement tools could be a solution for accelerating the genetic progress to achieve high enough selection intensity.

The overall pregnancy, lambing, and fecundity rates recorded in this study are comparable to the values reported by Najafi *et al.* (2014) in Ghezel ewes synchronized with CIDR and inseminated cervically with fresh diluted semen. Results of the present study were, however, higher than those reported by Olivera-muzante *et al.* (2011) who reported a pregnancy rate of 51% synchronized with two doses of PGF2 α seven days apart and inseminated cervically with fresh semen at 48 hours of post hormone administration. However, the current result was lower than values reported by Fornazari *et al.* (2018) who reported a pregnancy rate of 76.5% in Assaf ewes synchronized with MAP and FGA and inseminated cervically with fresh and chilled semen.

Significantly higher ($P < 0.05$) pregnancy and lambing rates were recorded in ewes inseminated with Dorper semen, compared to ewes inseminated with Menz semen (Table 4). Results of the present study were in agreement with the Ayele *et al.* (2016) and Shenkute *et al.* (2018) who reported lambing rate of 84.85% and 112% in Menz ewes sired by Menz and Dorper rams respectively. Results attained in the on-farm Menz pure breeding experiment were relatively less but not significant than on-station Menz pure breeding experiment. The reason might be due to differences in AI laboratory facilities as the on-station AI laboratory was fully equipped and have insemination yard designed for this purpose.

5.4. Pregnancy, Lambing and Fecundity Rates in Menz Sheep

Pregnancy, lambing, and fecundity rates were not varied across the four synchronization protocols in the current study (Table 5). The pregnancy and lambing rates under progestogen protocols was slightly higher but not significant than prostaglandin based protocols. This is in agreement with the findings of Gonzalez-bulnes *et al.* (2005) who reported fertilization rate

were not varied when estrus was synchronized with two dose of PGF2 α injection at 10 days interval or intravaginal sponges for 14 days. However, it is contrary to the findings of Olivera-Muzante *et al.* (2011) who reported superiority of progestogen in pregnancy rates over prostaglandin based protocols.

The pregnancy rate recorded in single injection of PGF2 α protocol in the present study are similar to the findings of Olivera-muzante *et al.* (2011) who reported pregnancy rate of 51% for ewes synchronized with two injection of PGF2 α seven days apart. Pregnancy rates recorded in the current study in two injection of PGF2 α , either seven or eleven days apart protocols are comparable to the findings of Allaoui *et al.* (2014) who recorded a pregnancy rate of 64% in Ouled Djellal ewes synchronized with FGA sponges. The present results under prostaglandin protocols were, however, higher than those obtained by Olivera-muzante *et al.* (2011) who reported pregnancy rate of 45% for ewes synchronized with two injection of PGF2 α in eight days interval, however, lower than those of Kumar (2015) who recorded a lambing rate of 71.4% in Nellore Jodipi ewes synchronized with two injection of 125 μ g with 9 in days interval.

Results attained in the current study under progestogen protocol was comparable to overall pregnancy rate of 76.5% recorded in Assaf ewes synchronized with MAP and FGA (Fornazari *et al.*, 2018). Contrary to the present findings under progestogen-based protocol, higher pregnancy and lambing rates were recorded by Ataman *et al.* (2006) in Merinos and Akkaraman crossbred F1 ewes (82%). However, pregnancy rate attained in the current study under progestogen protocol was higher than value reported by Olivera-Muzante *et al.* (2011) who reported a pregnancy rate of 71% in Australian Merino ewes synchronized with MAP.

Pregnancy, lambing, and fecundity rates were not significantly influenced by BCS and parity of ewes (Table 5). Studies in Suffolk ewes showed that BCS and parity of ewes were not significantly affected pregnancy and lambing rates (Fukui *et al.*, 2010). Findings by Palacín *et al.* (2012) indicated that pregnancy rate were not varied in ewes between two and five previous parturitions in Rasa Aragonesa meat sheep. Sanchez-davila & Ungerfeld (2012) indicated that pregnancy rates were statistically similar in multiparous and primiparous ewes (pregnancy rates of 59.6% vs 50.0% respectively). However, it is contrary to the findings of Solomon *et al.* (2016b) who reported the significant implication of parity and body condition of ewes on pregnancy rate.

5.5. Litter Size and Litter Weight in Menz Sheep

Litter size and litter weight did not vary across the four synchronization protocols (Table 6). The overall least squares means of litter size and litter weight obtained in the current study are in agreement with the findings of Rekik *et al.* (2016) in Menz ewes synchronized with progestogen, PGF2 α , and GnRH hormones and sired by Menz rams. The present results were lower than findings by Ayele *et al.* (2016) who reported 1.18 and 3.41 kg litter size and litter weight of Menz sheep sired by Dorper rams under natural oestrous respectively. The present results were, however, higher than those obtained by Shenkute *et al.* (2014) who reported a mean litter weight of 2.87 kg for Dorper x Unimproved Menz type sheep.

Litter weight was significantly varied among genotype (Table 6). Dorper x Menz cross breed lambs was significantly heavier compared to pure breed Menz lambs. Which is in consonance with lighter mean litter weight in improved Menz sheep reported by Rekik *et al.*, (2016) and

heavy litter weight of Dorper x Menz sheep (litter weight of 3.2 vs 3.41 kg respectively) reported by Ayele *et al.* (2016).

There was no a significant effect of BCS, parity, and sex of lambs on litter size and litter weight in Menz sheep (Table 5). Similar studies indicated that there was no significant effect of BCS, parity, and sex of lambs on litter weights in Norduz ewes and in Black Bengal goats in Tripura State of India respectively (Karaku and Atmaca, 2016; Halдар et al., 2014). The present results were, however, contrary to the finding of Ayele *et al.* (2016) who indicated parity had a significant effect on litter size and litter weight. Same author showed that sex of lambs had no significant effect on both litter size and litter weight in Menz sheep. Moreover Sanchez-davila *et al.* (2015) reported that there was a significant effect of parity and year of lambing on litter size and litter weight in Saint Croix hair sheep.

5.6. Genotype and Ejaculation Frequency on Semen Quality Parameters

The results of the present study (Table 7) showed that ejaculation volume and concentration of spermatozoa was higher ($P<0.05$) in Dorper rams compared to Menz rams which is in consonance with that of Shenkute (2018). Same source indicated that Dorper rams were relatively higher scrotal circumference related to Menz rams. Scrotal circumference had a significant positive correlation to ejaculation volume, sperm motility, and percentage of live spermatozoa in rams (Maksimović *et al.*, 2016). Findings by Etim (2015); Kheradmand *et al.* (2006) also indicated that total mass of sperm producing tissue, live sperm count and sperm cell normality were positively correlated to the size of scrotal circumference. The effect of breed upon scrotal circumference and sperm quality in domestic animals is also reported by other scholars (Latif *et al.*, 2009; Lemma & Shemsu, 2015; Quezada-casasola *et al.*, 2016).

However, study by Tabbaa *et al.* (2006) indicated that scrotal circumference was poorly correlated to ejaculation volume, concentration of spermatozoa, mass motility and sperm abnormality percentage in Awassi rams. No significant difference was observed in semen mass motility among genotypes. This is in close accordance with the findings by (Shenkute, 2018) who reported that no significant difference was observed in mass motility among Dorper and Menz rams.

Ejaculation volume was varied across ejaculation frequency (Table 7). Ejaculation volume was higher ($P < 0.05$) in initial ejaculate compared to consecutive ejaculates. This is in agreement with the findings of Yotov *et al.* (2009) who reported ejaculation volume decreased gradually with increased in the ejaculation frequency in Pleven Blackhead rams. There was no significant difference in mass motility across ejaculation frequency. However, The result of the current study are in contrary with the previous findings of Bahadur *et al.* (2016); Yotov *et al.* (2009) who observed higher semen motility in the consecutives ejaculates compared to initial ejaculate. Findings by Nel-themaat *et al.* (2006) indicated that first ejaculate was lower mass motility compared to second and third ejaculates in Gulf Coast native rams respectively. The reason for no variation in semen mass motility among ejaculation frequency in this trial might be due to semen was collected from rams trained to serve AV for a week. Semen was collected from rams once per day in non-consecutive days for a week before the commencement of actual collection. Studies by Ari *et al.* (2011); Santolaria *et al.* (2011) indicated that semen collection two to three times per week on non-consecutive days improve semen motility of first ejaculate for artificial insemination.

6. SUMMARY AND CONCLUSION

6.1. Summary

CBBP is commonly not effective since large number of breeding rams were selected resulting in low selection intensity and thus slow genetic progress. However, AI enables sheep farmers in the CBBP to accelerate the rate of genetic improvement by maximizing the number of offspring from a desirable fewer best rams.

Large number of both male and female high producer exotic sheep breeds was imported in Ethiopia for crossbreeding programs. However, by maintaining minimum effective population size (N_e) AI could be a solution to import fewer exotic genotype than natural breeding.

The current work was conducted at Debre Birhan Agricultural Research Center and Menz community based sheep genetic improvement program villages, with the aim to evaluate success of estrus synchronization and artificial insemination in Menz sheep.

A total of 166 ewes were kept in Debre Birhan Agricultural Research Center and Menz community based breeding program villages for pure breeding experiment and was allocated to one of three protocols randomly. The animals were blocked based on parity and body condition score. Accordingly, animals were assigned to one of the protocols randomly. Thus Protocol one ($n=60$) single injection of $\text{PGF}_{2\alpha}$; Protocol two ($n=54$) double injection of $\text{PGF}_{2\alpha}$ 7 days apart; and Protocol three ($n=52$) double injection of $\text{PGF}_{2\alpha}$ 11 days apart in on-station and on-farm experiments respectively.

A total of 83 Menz ewes kept in Debre Birhan Agricultural Research Center in Dorper x Menz crossbreeding experiment and were assigned into four protocols randomly. Same criteria were

used to block the animals. Then the animals were subjected to one of the four protocols randomly. Protocol one (n=20) single injection of PGF2 α ; Protocol two (n=24) double injection of PGF2 α 7 days apart; Protocol three (n=19) double injection of PGF2 α 11 days apart; and Protocol four (n=20) intravaginal progestogen sponges, containing 45 mg of FGA, followed by administration of PMSG at sponge withdrawal.

Fixed time cervical insemination was performed in estrous ewes 48-51 hrs following last PGF2 α and PMSG administration with 0.25 ml fresh diluted Dorper and Menz semen for Dorper x Menz crossbreeding and Menz pure breeding experiment respectively.

Overall, across the four synchronization protocols 77.5% of ewes exhibited overt sign of oestrous within 48 hrs of hormone administration. There was no significant difference in percentage of ewes exhibiting overt signs of oestrus within 48 hours of hormone administration across protocols, management, and parity. However, ewes that had between 2.5 and 3.0 BCS were significantly higher responsive to hormonal treatment compared to ewes that had 2.0 body conditions score.

The overall pregnancy, lambing and fecundity rates were 59.1, 58.0, and 98.2% respectively. There were no significant differences in pregnancy, lambing, and fecundity rates between ewes due to difference in synchronization protocols, BCS, and parity. However, ewes inseminated with Dorper semen had significantly higher pregnancy and lambing rates compared to ewes inseminated with Menz (73.5 and 72.1% vs 51.2 and 50.4% respectively).

The overall least squares means of litter size and litter weight obtained in the current study were 1.04 and 3.0 kg respectively. There was no significant difference in litter size and litter weight across protocols, BCS, parity and sex of lambs. However, there was significant

difference in litter weight among the genotypes. Dorper x Menz lambs had heavier litter weight compared to pure Menz lambs (3.3 vs 2.8 kg respectively) however, no statistical difference in litter size among genotypes was observed.

Ejaculation volume and concentration of spermatozoa were higher in Dorper rams compared to Menz (0.92 ml and 6.04×10^9 vs 0.62 ml and 4.11×10^9 respectively). There was significant difference in ejaculation volume across ejaculation frequency. Ejaculation frequencies had significant effect on ejaculation volume. The volume was higher in first ejaculate compared to second and third ejaculates respectively (0.82, 0.61 and 0.64 ml respectively). Moreover, results pertaining mass motility and spermatozoa concentration across ejaculation frequencies were not statistically significant.

6.2. Conclusion

The current work strongly indicated that oestrous could be effectively synchronized for achieving both planned and concentrated lambing in Menz sheep. This study revealed that no statistically significant difference was observed in the use of either double dose administration of PGF2 α or intravaginal progestogen sponges over a single dose PGF2 α administration.

The present result also revealed that artificial insemination can be thought possible as a strategy in sheep genetic improvement programs. To boost the rate of genetic gain AI also could be a solution to import fewer exotic genotypes and allows us to producing large number of crossbreed lambs from imported breed with in few years.

7. RECOMMENDATIONS

To achieve planned and concentrated lambing in Menz CBBP areas, oestrous synchronization using single injection of PGF2 α (1 ml Enzaprost®) would be most feasible.

For successful oestrous synchronization application ewes that have a body condition score of 2.5 or above should be considered.

In addition to the use of fresh semen AI, the success rate of AI with chilled and frozen ram semen in Menz sheep needs to be further investigated.

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9. APPENDIX





Figure 1: Pictures of lambs born with AI at DBARC

BIOGRAPHICAL SKETCH

The author of the thesis was born at Alem Ketema, North Showa Zone, Ethiopia on April 15, 1989 G.C. He attained his elementary and high school at Alem Ketema primary and Arbegnoch secondary school from 1995 to 2004. He thereafter attained his Preparatory school at Debre Birhan Haile Mariam Momo School from 2005 to 2006. He studied BSc (Animal, Rangeland and Wildlife Sciences) at Mekelle University from 2007 to 2009 G.C. From October 10, 2009 to July 7, 2011 he was employed on Angolelana Tera Agricultural office as Animal sciences expert. He was then working on Debre Birhan Agricultural Research center as Assistance researcher from July 8, 2011 till October 12, 2016. The author was registered in October 2016 to pursue Master of Science in Animal Breeding and Genetics at Hawassa University.