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COLLEGE OF NATURAL SCIENCES AND COMPUTATIONAL SCINCENCES

DEPARTMENT OF MICROBIAL CELLULAR AND MOLECULAR BIOLOGY

MORPHOLOGICAL AND GDF9 GENE POLYMORPHISM OF INDIGENOUS GOAT POPULATIONS IN DARRA DISTRICT

MSc Thesis

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This is to certify the thesis prepared by Ejigayehu Siraj Muhmmed entitled:"**MORPHOLOGICAL AND GDF9 GENE POLYMORPHISM OF INDIGENOUS GOAT POPULATIONS IN DARRA DISTRICT**" submitted in fulfillment of the requirements for the Msc Degree in Applied Genetics complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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As member of the Board of Examiners of the M.Sc. Thesis Open Defense Examination, we certify that we have read, evaluated the thesis prepared by Ejigayehu Siraj Muhmmed and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Applied Genetics

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LIST OF ACRONYMS AND ABBREVIATION

BL	Body Length
BW	Body Weight
CCP	Coat Color Pattern
CCT	Coat Color Type
CG	Chest Girth
CSA	Central Statistical Agency
FAO	Food and Agricultural Organization
G	Glossy
GDF9	Growth Differentiation Factor 9
GLM	General Liner Model
HT	Hair Type
P	Patchy
PCR	Polymarase Chain Reactions
PL	Plain
PW	Pelvic Width
RH	Rump Height
RL	Rump Length
SH	Straight Hair
SNP	Single Nucleotide Polymorphism
SP	Spotted
SPSS	Statistical Package for Social Science
SS	Smooth Hair

ABSTRACT

Goats among the livestock species, are considered the most prolific ruminant which are adapt in different farming areas and agroecological zones in highland, subhumid, semiarid, and arid environments. Characterization of genetic resources is a prerrquesit to design sustainable genetic improvement program. Therefore the objective of the study was to assess morphological variations and GDF9 gene polymorphism in indigenous goat population in darra district North Shoa Zone of Oromia Regional State. A total of 336 goat population were selected from seven kebele those have single, twin, triple litter size using stratified purposeive sampling method. Body weight and linera body measurement were taken from 336 goats and blood samples were also collected from 150 goats only. Genomic DNA was extracted using salting out procedure. All samples were genotyped using GDF9 gene primer pairs. The most frequent coat color pattern observed was plain, spotted, red and brown were observed coat color types in the study area. The analysis of variance showed a significant variation ($p < 0.05$) in morphological traits among does in different agroecolgies,location with different litter size. Goats in highland agro ecology showed better body weight and linear body measurmetns compared to lowland agroecolgy. Goats having single litter size showed low value in most measuremtns compare to twin and triplet. However, there was no variation observed among goats of twin and triplet for except pelvic length. GDF9 locus was polymorphic in twin and triplet litter sizes and showed two alleles (462, 478) and three genotypes (462/462, 462/478 and 478/478) that segregated with different frequencies Genotype 462/462 had high frequence (0.47) followed by genotype 462/478(0.43) in twins where as genotype 462/478 (0.47) showed higher frequency followed by genotype 462/462 (0.38) in triplet. The expected hetrozygosity (H_E) ranged from 0.43 in triplet to 0.47 in twin litter size. The closest genetic relationship was found between goats having twin and triplet (0.09), while goats of singleton and their triplet counterparts were more distant apart (0.16).Significant ($p < 0.05$) and positive association of GDF9 genotypes with litter size and linear body measurements were observed. Morphological variation and genetic variation at GDF9 locus could be an opportunity to design cost effective breeding strategy. However,the current result is prilimnary, large nuber size at different location should be considred to subsatatiate the current result.

Keywords: Fecundity gene, Linear body measurement, Litter size, Morphology and Phenotype

1.INTRODUCTION

1.1. Background and justification

Sub-Saharan African food production methods rely primarily on the utilization of animals that have adapted to their environment. Those animal are crucial to human agriculture,culture,and economy. Ethiopian economy is depending on agriculture. Livestock is one of an integral part of agriculture and plays vital role in reducing poverty, enhancing food security, contributing to the increase of the national income, exports and foreign revenue (Shapiro *et al*., 2015). Goats among the livestock species, are considered the most prolific ruminant which are adapt in different farming areas and agroecological zones in highland, subhumid, semiarid, and arid environments (Getent Mekuriaw, 2016). Goats were an excellent food resource because to their remarkable adaptability,moderate size, and hardiness(Amills *et al*., 2008). Since the comparatively low input needs and high anticipated output, goat farming is particularly essential (Brahmi *et al*., 2012). Additionally,goat meat is a better choice for your health than beef or chicken because it has lower levels of calories, fat, and cholesterol. Goats meat are better than other red meats because it is more dietary and abundant in minerals, unsaturated fats, and amino acids. The goat meat is hypoallergenic as well, making it appropriate for child nutrition (Bhattarai, 2015; Becic, 2017).

Demand for goat meat increased from time to time in most developing countries (Mazhangara *et al.*, 2019, FAO, 2020) which is affected by urbanization, consumer preference and increasing awareness of goat meat quality (Palmer *et al*., 2022). The same trend is followed in Ethiopia goat production play significant role for Ethiopian economy it provides cash income, meat and skin production for the livelihoods of smallholder farmer. Additionally, it serves as the main source of work and income for women, children and elders in low input production system (Melkamu Bezabih and Gebreyohannes Berhane, 2014) and contribute for the export market as well (Tekeba Eshetie *et al*., 2018). Besides, large livestock population, huge young rural workforce and close ties to one of the world's largest meat markets the Middle East created an opportunity for the goat producer in Ethiopia. However the indigenous goat genetic resources are not fully exploited. Understanding the diversity and improving the existing productivity have a paramount importance for the livestock industry in Ethiopia.

Farmer must improve goat productivity by improving goat genetic resources since their meat better for human health goats are distributed over all types of ecology with more concentrated in the tropics and dry zones of developing countries. About 617 million goats in the world 97.3 % are found in the developing world 65.9 % are found in Asia, 27.4 % in Africa, 3.5 % in Europe and 3.0 % in Americas. The number of dairy goats in the world is 191 million goats, 47.7 % of them are in the 25 least developing countries (FAOSTAT, 2012). Goat population in Ethiopia accounted about 45.7 million from these about 70.49 percent of goat are female and 29.51 percent are male. About 99.97% of the Ethiopian goats are indigenous (CSA, 2022). They are phenotypically divided into four groups and twelve different major breeds (Solomon Gizaw *et al.,* 2010). However,only eight goat breeds have been identified based on farm animal breed characterization and identification (Ethiopia Biodiversity Institution, 2016). Keffa, Abergalle, Afar, Highland goat, Eastern and south Easter goat, Gumez, Arsi-bale and woyto-Guji goat are among those eight goat breed types. Ethiopia has a high goat population, however despite this,the country's and region's economic output is very low. This may be because of a variety of factors, including poor nutrition and the prevalence of various diseases, feed shortage, environmental condition, inferior genotype, inadequate breeding practices and breeds, as well as a lack of comprehension of the production system as a whole (Tesfaye Tsegaye, 2009).

Local goat breeds were replaced and crossed with imported goat breeds including Anglo-Nubian, Saanen and genberg brought by various organizations at various times due to their lower productivity. Indiscriminate crossbreeding of native goats can resulted in genetic erosion, loss of genetic variety and reduction of adaptive value for effective utilization of the existing adapted goat genetic resources (Halima Hassen *et al.,* 2012). Different native goat breeds each have a unique relative advantage in their natural habitat. Phenotypic selection does not influence the economy of farmers. Focusing on the development of reproductive qualities will have a long-term effect on goat production's profitability (Pramod *et al.,* 2013). In order to choose genetically superior animals for increased productivity and disease resistance it can be necessary to solve the issues with traditional selection through the use of molecular data for trait selection (Singh *et al.,* 2013). Animals that produce twins contribute more than 1.5 times as much to the production of meat as those that only generate one child per kidding(Ahlawat *et al.,* 2016)

Molecular genetics discovered individual genes or candidate genes with substantial effects on these traits of economic importance. The complicated quantitative feature of litter size involves several genes and loci as well as external influences,maternal effects such maternal, age and intrauterine environment. Modern genetics faces a different factors in identifying complete candidate genes and loci linked to prolificacy/litter size.Due to many genetic influences on the complicated quantitative litter size trait one or a few genotype polymorphisms in genes related to reproduction,such GDF9 may be associated with the characteristic. One of the most significant and commercially important reproductive traits in goat farming is litter size. In many goat breeds growth differentiation factor 9 gene (GDF9 gene) polymorphism and its relationship to litter size have been investigated (Feng *et al.*, 2011; Chu *et al.*, 2011; Abodoli *et al*., 2013). However, there is limited information on growth differentiation factor 9 gene (GDF9) relationship between polymorphism and litter size and body measurements for Ethiopian goats except some information on polymorphism of KISS1 gene (Getenet Mekuriaw *et al.*, 2016).

The information from association study would help for marker assisted selection hence identifying gene polymorphism of major effect on litter size is also a prerequisite which offers the opportunity to improve reproductive efficiency through utilizing them in breeding programs. It also help as baseline information in designing sustainable genetic improvement strategy for commercialization of goats' production. The study's objective was to determine the growth differentiation factor 9 gene (GDF9 gene) polymorphism and its association to the linear body measurement and litter size of local goats. Profitability of goats production is depends on litter size of does (female). However,reproductive traits have low heritability ranging from 0.1 to 0.2 (Sun *et al.*, 2010) .Because phenotype-based selection is frequently ineffective and slow (Abdoli *et al*., 2013). Selection of breeding does based on genotype related with litter size in goats would be an effective way of improving the productivity of does through reducing stocking rate, labor and flock nutritional requirements. Multiple genes known as fecundity genes have an impact on litter size in domestic species (Gootwine, 2020).

1.2. Statement of the problem

Though there are large population of indigenous goats in Ethiopia the demand for goat meat and live animal for international and domestic markets are increase to meet this huge market demand the general productivity of indigenous goat is currently too low which is estimated about 8 kg off take rate (Sebesibe, 2011) which could be due to low reproductive efficiency,disease prevalence and poor nutrition, Seasonal and environment factors, genetic factors, hormonal factors and also due to increasing human population growth, income and expansion of urbanization. Improving the reproductive efficiency of goat is important to increase the efficiency of kids production (prolificacy) and consequently goat meat (Anous *et al.,* 2009). However improvement of reproduction efficiency by traditional selective breeding methods is difficult to sustain human interest due to the low heritability only about 0.152 and long reproductive cycle (Zhang *et al.,*2009; Sun *et al.,* 2010; Wang *et a*l., 2012).

Molecular breeding through marker-assisted selection is for this reason the only effective way to alleviate the inefficiency and long cycle length of traditional breeding was improved by molecular breeding which can shorten the time of new improved variety from 8-10 generations to 2-3 generations (Wang *et al*., 2012). Due to fecundity genes which is GDF9 that belonging to transforming growth factor (TGFβ) superfamily has major effect on ovulation rate,litter size and reproductive performance of different goat breeds (Ran *et al.*, 2018). Fecundity gene is important for some economically important domestic animals like goats. Goat meat is a healthy alternative to beef and chicken because of its lower calorie, fat and cholesterol totals. The goat meat has numerous benefits compared to other red meats. It is more dietetic, rich in amino acids, minerals and unsaturated fats. In addition, the goat meat is hypoallergic, which makes it suitable for child nutrition (Bhattarai, 2015; Becic, 2017). By using new technology that focus on trait to understand its mechanism(Gebson *et al.,* 2015; Zhang *et al*., 2015) and to develop related biomarker for further animal breed (Wang *et al.,* 2015).

The identification and use of major fecundity genes in goat production would enable an increase in reproductive traits and genetic improvement and also, an increase in ovulation rate and litter size would result in higher productivity of female goat and meat production would become more

efficient because more kids are produced per female goat. Even though different factors such as environment, genetic, avalablity of feed, disease, season affect the productivity of indigenous goat population in the study show the association of linear body measurement and GDF9 gene on litter size show they have positive correlation so the current study provide a baseline information to design sustaianable breeding program to improve reproductive performance of goat.

1.3. Research questions and hypothesis

1.Is there variation among goat/does with different litter size?

2.Is there GDF9 gene polymorphism among goat/ does with different litter size?

Research Hypothesis. There is genetic diversity in indigenous goat managed in high land and low land agro ecological setup. There is fecundity gene which is growth differentiation factor 9 gene polymorphism among indigenous goat population.

1.4. Objective

General Objective

To assess morphological variations and GDF9 gene polymorphism in indigenous goat indigenous goat population in the study area

Specific Objectives

1. To assess the morphological variations among indigenous goat population in the study areas
2. To assess the GDF9 gene polymorphism in indigenous goat population with differen**t** litter size

1.5. Significance of the study and expected outcome

The current study provides baseline information on genetic diversity of indigenous goat across highland and lowland agro ecology, fecundity gene growth differentiation factor 9 gene (GDF9gene) polymorphism and its effect on reproductive performance of indigenous goat on those have twin and triplet ability,the effect of morphological variation,environmental factors on

prolificacy/litter size of indigenous goat breed in the study area. The quantative and qualitative measurement the important parameter to select goat which has better reproductive capacity and their association with litter size/prolificacy with fecundity gene which is growth differentiation factor 9 gene (GDF9 gene). The information provided would be expected to have valuable advantage that helps in understanding indigenous goat type that is used in delivering required effort to enhance the production and productivity of breed at large scale. The information generated in from this study help researchers, students and different organizations working on genetic diversity genetic improvement and on contribution of other factors on prolificacy/litter size of goats in the study areas because productive capacity of goat can't bring radical change by selective breeding method.

1.6. Scope and limitation of the study

The study was restricted to Darra district, which is one of the districts in North Shoa Zone Oromia National Regional State Ethiopia. The study focused on only morphological variations and GDF9 gene polymorphism in indigenous goat population in darra district to know their polymorphism of fecundity gene such as GDF9 the effect of morphological variation and agro ecology on the prolificacy/litter size of indigenous goat. The limitation of this research also only focused seven Keble's three Keble lowland and four highland agro ecologies which may not capture the good situation at zonal and regional level.

2. LITERATURE REVIEW

2.1. Goat production and genetic resources in Ethiopia

Goats are regarded as one of the first animals that humans domesticated Historical and archcological evidences indicate that the domestic goats could have been domesticated from two wild Capra species (Capra.aegagrus and Caprafalconen) (Epstein, 1971). According to regional, economic, social and environmental factors they vary in presentation and concentration. In tropical and subtropical areas, goats are the most polific domesticated ruminant species (Hagos Abraham *et al.,* 2018). The populations of goats may help rural households that increase livestock on these continents improve their standard of living.By 2050, there can be a need for around 73% more meat and 58% more dairy products to feed the world's expanding population, which is projected to grow from 7.2 billion to 9.6 billion people (FAOSTAT, 2016). All administrative region of the country have a large population of indigenous goat breeds which likely developed through a process of artificial selection that prioritized adaptation and survival over reproduction.

The most important goat feed source in the area of study were grasses, shrubs and root leaves that were found in a natural pasture and Acacia trees, euphorbia, (fodder trees), Sesbania, trelucer, legume trees with pods were the other important goat feed source. Even though, there were a difference in priority and indices value across agro-ecologies in feed resources both during wet and dry seasons. Natural pasture, fodder and legume trees were major feed resources for goat at Dera districts(Wondimeneh Simeneh, 2019). Natural pasture is common feed resources for goat during wet season in different parts of the country (Alubel Alemu, 2015)

Goats are characterized by their need for simple food and management,their capacity for twin birth, their high rate of feed conversion, their early sexual maturation, their long lifespan, their ability to perform well as a breeder, their ability to feed on rations that are not consumed by other animals and their low price in addition to their variability in production, which is why they are known as the cow of the poor. A farmers in various production systems may choose different traits and they may also employ a variety of techniques depending on the agro-environments in which they operate (Solomon Gizaw *et al,*.2010). Knowing which traits are essential to farmers in their agro-ecosystem and how to incorporate them into the design of sustainable breeding programs can be

learned from studying farmers' trait preferences (Yadeta Nemebergaga, 2016). When choosing female goats farmers use a variety of characteristics in eastern Ethiopia, female goats or ewes were chosen based on appearance,coat color and lamb survival (Netsanet Zergaw, 2014). The selection criteria used for male goats are different from those used for breeding females (Yadeta Nemebergaga, 2016). For males, tail type, color and height are given the most emphasis for selection (Tesfaye Alemu, 2008). Additionally breeding females with long lambing intervals, black color, old age and poor condition are also culled. Using the offspring of a chosen parent (buck or doe) and body size, growth rate and libido (sexual urge) priority selection criteria is the most typical method of choosing goats as parents for the upcoming generations (Hagos Abraham *et al.,* 2017).

There are approximately 45.7 million heads of goats spread across the different agro-ecologies ranging from cool alpine climate of the mountains to the arid pastoral areas of the lowlands (CSA, 2022). Table 1 demonstrates a comprehensive survey of goats in Ethiopia and Eritrea identified 14 clusters of breeds based on morphological characteristics (FARM-Africa, 1996). These include the Somali family (short-eared and long-eared Somali), the Nubian family (Nubian and Barka), the small Rift Valley family (Abergelle, Worre, Afar, Arsi-Bale and Woyto-Guji) and small East African family (western highland, Kefa, central highland and West lowlands goats.

Table 1. Goat breeds of Ethiopia and their geographical distribution

Family name	Breed name	Vernacular name	Distribution
Nubian	Nubian	Barka, Begayt	West Tigray
Rift valley	Afar	Adal, Denakil	Afar area, Western and Northern
	Abergelle	NA	Hararghe, Tigrayregion, Wag Himr and East Gondar along the Tekeze river
Somali	Woyto-Guji	Woyto Guji, Konso	South Omo, Southern Sidamo and Wolayta
	Hararghe Highland	NA	Hararghe
	Somali short-eared	Denghier	Northern and Eastern Ogaden
	Somali with long ears	Denghier, Digodi Melebo	Ogaden, Low land of Bale and Borena

African	Western Highland	NA	Highland of South Gondar, Gojam, Wollega and West Shoa
	Western Lowland	Gumuz	
	Keffa	NA	Along the area bordering the Sudan Highlands and Lowlands of Keffa and South Shoa Zone

Source.FARM Africa, 1996 and DAGRIS, 2006, NA= Not available

2.2. Reproductive performance of indigenous goat

Fecundity a factor in reproductive performance that is significant to the goat industry has attracted a lot of attention as a result of the possibility that small improvements to reproductive traits, such as increasing litter size could result in significant increases in profit (Tao *et al.*, 2011). As molecular genetic technologies have advanced numerous methods have been developed to find evidence of selection various candidate genes and genetic loci have been identified (Guan *et al*., 2016). Reproductive performance becomes the most crucial characteristics for animal production when meat production is the primary goal (Alkass *et al.*, 1982). Fecundity is expressed as a percentage of the number of kids per female doe or per 100 females per year, the mean number of twin births, the number of twin births relative to the total number of female births, and the percentage of some key indicators for reproductive performance (Alkass *et al*., 1982).

Reproductive parameters' effects on the strength of selection have a significant impact on genetic progress. Planning a workable (viable) breeding plan requires adequate understanding of the reproductive abilities of the local breeds. However nothing is known about the reproductive characteristics of local goat breeds (Yoseph Mekasha, 2007). The factors that have the greatest economic ramifications for small ruminant reproductive performance are age at puberty and at the first kidding as well as the interval between kiddings and the size of each kidding. According to genotype, nutrition, season and other environmental factors male goats develop at different rates when it comes to reaching puberty (Abi-Saab *et al*., 1997). Goat breeding goals and selection criteria may be significantly influenced by the poor reproductive performance levels seen in communal production systems (Kogsley *et al*., 2006). Both genetic and environmental factors have an impact on these indicators.

Additionally well known as seasonal breeders are goats. A number of variables including latitude, climate, breed, physiological stage, presence of the male (buck effect), breeding system and photoperiod, affect the start and length of the breeding season. Goats breed during the in autumn and winter in temperate climates. Goats are often constant breeders in tropical areas but when food is scarce, anestrous spells are common. The reproductive trait is a fitness trait that has to do with viability and reproduction. The litter size is included.numerous factors affect the reproductive characteristic which varies, breed of goat, location rearing, season of conception, season of kidding, kind of dam's birth, age of kidding and nutrition and health care are a few significant elements that determine reproductive features. Location has been identified as a significant factor influencing the first conception, kidding, postpartum estrus, and gestation length (Kolachhapati *et al*,. 2012).

Quantitative and qualitative trait of goat population in different location and GDF9 gene has significant effect on litter those factors increase reproductive capacity of goat. The reproductive characteristics of the native goat of Nepal are also influenced by the season during which conception occurs.(Parajuli *et al*., 2015). The season of kidding is also high importance for the reproductive trait (Parajuli *et al*., 2015). When there is enough green forage available, does (female goats) reach sexual maturity earlier which results in active release of reproductive hormones and shorter gestation periods throughout the summer.

2.3. Reproduction efficiency and litter size of goat

It is a trait that is influenced by the quantity of fertilized oocytes and dependent on ovulation rate. The likelihood of larger litters increases with a greater ovulation rate because more oocytes will be available for fertilization during the estrous (Drouilhet *et al*., 2013). Increase in litter size,or prolificacy is a crucial economic trair for farm animals. Multiple genes known as fecundity genes affect prolificacy in domestic animals genetically (Drouilhet *et al*., 2013). Prolificacy is closely associated with follicular development and ovulation rate. Ovulation rate and litter size are crucial reproduction traits with high economic value (Notter, 2008). It is controlled by various factors like

Single gene mutations, age, environmental change and season, nutrition, hormonal factors, fertilization rate, embryonic and foetal development,Genetic factors.

2.4. Characterization of Goat gentic resources

2.4.1. Morphological diversity of indigenous goat

Phenotypic characterisation is an important early step in breed identification. The underutilization, replacement and dilution of indigenous goat breeds through cross breeding may occur despite their local adaptation to environmental limits because to a lack of knowledge about genetic resource features. Variation in observable trait which should be compared among breeds at a similar physiological stage (age) for evaluative purposes may be revealed the genetic variety of goats. Selecting superior animals for reproductive purposes is crucial. However, there is a lacking of such a comparison for goat breeds raised in tropical regions with intensive husbandry in general and for Ethiopian goats in particular since there is little information available on the reproductive trait of native goat breeds this is especially true of male reproductive trait which were largely disregarded in the breed characterisation undertaking (FARM-AFRICA, 1996).

Phenotypic characterization includes the morphological description of the animals and the phenotypic trait in which they are produced (FAO, 2011). External characteristics,such as goat color, horn type and shape, beard, ear type and shape, linear body measurements (such as body length, heart girth, ear length and tail length), production traits (such as carcass weight, milk yield) and reproductive characteristics (such as age at first service, number of litters) are all included in the phenotypic description of breeds (FAO, 2012). One method of population identification used in a particular geographic area is the physical description of the population (Galal *et al*., 1996) (Table 2). Morphological characterization also a method which used to select better production trait because most linear body measurement has significant effect or association with litter size of goat population. Goat which has twinage and triplet has highest body weight, body length, chest girth.

Table 2 Distribution and morphological characteristics of Ethiopia's five goat populations

Population	Area of distribution	Physical characteristics	Source
Gondar	Highlands and lowlands of Gondar	adult body weight (33.95 kg), height (71.02 cm), chest breadth (15.48 cm) and pelvic width (12.2 cm)White coat color (21.66%) and its mix with other coat colors (55.09%) can be used to describe the population	Alubel Alemu, 2015
Arsi-Bale Highland	Highland of Arsi and Bale	Bale Plain coat color (black and white) is the most common pattern followed by patchy and infrequently patterned.The highland goat's predominant coat colors included black with patches,white with patches,red,brown and grey long,wavy and glossy hair (mean=13.8 cm) and white with patches.	Getinet Mekuria *et al*., 2016
Arsi-Bale Lowland	Lowlands of Arsi, Bale, Hararghe and mid rift valley of Ethiopia	Coat color patter that was predominantly plain (white) and glossy,wavy and gray in c Coat colortype.	Hussein, 2015
Wo`yto-Guji	South Omo, Southern Sidamo and Wolayta	For both sexes, the predominant coat color patterns were patchy (67.4%), plain (24.9%) and spotted (7.6%). Black, brown, and white are common colors for plain	Biruh Tesfahun, 2013

2.4.2. Ethiopian indigenous goats' body weight and linear body parameters

The composition of the carcass can be indirectly determined by body measurements which are indicators of skeletal development (Attah *et al.,* 2004). Preliminary classification findings from 1996 there is variation in native goat breeds' body weight traits (Table 3) (FARM Africa's, 1996). Goats are essential for attaining the required market body weight with appropriate carcass composition. The use of body weight either in assessing animal productivity at the farmer level or in deciding cost at the majority of marketing channels in the country is almost absent because they are important meat animals in the tropics growth performance and body weight changes this is

mostly due to unavailability of scales at farmers level. Through regressing body weight on a number of easily measured physical parameters the practical difficulties of measuring body weight has increased reliance on weighing animals without a scale(Joseph Mekasha, 2007)

Table 3 Linear body measurements of adult females of Ethiopia's indigenous goat breeds.

Breed	Measurements (Mean + SD)		
	WH (cm)	BW (kg)	CG (cm)
Nubian	70.1 ± 3.4	34.1 + 5.4	74.3 + 3.8
Begayit	67.9 + 4.3	33.8 + 5.3	73.9 + 4.8
Afar	60.9 + 3.3	23.7 + 3.4	67.4 + 3.8
Abergelle	65.0 + 2.8	28.4 + 3.5	71.2 + 3.8
Arsi-Bale	66.1 ± 3.5	30.4 ± 4.5	74.9 ± 4.0
Woyto-Guji	66.4 ± 3.5	28.8 ± 5.0	72.5 ± 4.2
Woyto-Guji	62.5 ± 3.5	29.1 ± 4.5	72.8 ± 4.5
Somalishort-ear	61.8 ± 4.1	27.8 ± 6.0	70.4 ± 4.7
Somalilong-ear	69.4 ± 3.3	31.8 ± 5.4	74.4 ± 4.0
Middle Highland	67.9 ± 3.2	30.1 ± 5.4	74.1 ± 4.4
Western Highland	70.8 ± 4.7	33.0 ± 6.0	75.8 ± 4.5
Western Lowland	63.5 ± 3.8	33.9 ± 6.9	75.9 ± 5.2
Keffa	66.7 ± 4.0	28.2 ± 5.2	72.2 ± 4.5

BW, WH, CG, and SD stand for body weight,wither height,and chest girth, respectively.Adapted from FARM Africa (1996)

2.5. Molecular characterization of goat genetic resources

The similarity of the genetic material carried by populations and the genetic variation they possess can be determined by looking at the structure and function of genes at the molecular level in a breeding population. To determine the relationship among populations and their genetic variation or polymorphisms various approaches have been developed. These techniques include immunological, molecular (DNA hybridization, RFLP, mtDNA, microsatellites and SNP) and biochemical polymorphisms.(Hase and Rickwood, 1990). Molecular data have grown more important for characterizing genetic diversity since the 1990s (Groeneveld *et al*., 2010). Examining an organism's genetic makeup at the DNA, protein and other small molecule levels is known as molecular characterization. Estimating the degree of genetic diversity within and between breeds population structure and genetic differentiation aimed at genetic improvement and genetic resource conservation is important (Toro *et al*., 2011).

Molecular characterization is important to know economically important traits, breed history, in order to guide breed development, utilization and several potentially fecundity genes have been identified in the genetics of prolificacy in goats (Ahlawat *et al.,* 2015). In order to identify the preferred loci underlining characteristics that are significant for breeding it is important to use molecular markers when creating appropriate breeding strategies for economically advantageous animal species,the analysis of genetic variation is essential (Maudet *et al*., 2002). Different ovulation rates and litter sizes are affected differently by fecundity gene mutations. Some mutations have cumulative effects,whereas others make homozygous people infertile. In different breeds of goats ovulation rate and litter size are affected by various mutations in the genes of the transforming growth factor (TGF) super family. One of the important genes is called Growth Differentiation Factor 9 (GDF9). In numerous studies on various goat breeds across the world genetic polymorphism and prolificacy have been shown to be associated (Jalbani *et al*., 2017).

The correlation between GDF9 genetic variation and litter size in several goat breeds (Feng *et al*., 2011). The Teddy goat is known for its ability to survive in harsh environments, its rapid growth rate and its high prolificacy whereas the Beetal is a heritage goat and a symbol of Pakistan and Indian (eastern) Punjab and is used mostly for milk and meat (Khan *et al*., 2016). In Teddy goats,

the twinning rate is greater than 70%, compared to 50% to 55% in Beetal goats (Nawaz, 2013; Muhammad *et al.*, 2015). So there is genetic variations among GDF9genes. In Ethiopia, few researches have been conducted in genetic diversity of goats using microsatellite markers and mtDNA analysis and makes difficult to implement the above points (Ajmone-Marsan *et al.*, 2014).

Genetic diversity of some indigenous Ethiopia goats were studied using 15 microsatellite markers even though to get more accurate data and carry out comparative genome analysis for various breeds it recommended to use 30 microsatellite markers for molecular characterization of farm animal genetic resources (Tesfaye Alemu, 2004, FAO, 2011, Halima Hassen *et al.,* 2012). Furthermore microsatellite is not helping to determine quantitative trait loci that can be used for designing breeding program using marker assisted selection (Solomon Gizaw *et al.,* 2013). In order to improve genetics and increase litter size and reproduction efficiency it may be essential to identify polymorphism and DNA markers associated with reproductive characteristics (Ghaffari J *et al.,* 2009).

2.6. Fecundity gene polymorphism in goats

Fecundity is potential to reproduce offspring which influenced by female ovulation, age, menopause, environment and genetics. Fecundity gene is important for some economically domestic animals by using new technology focus on trait to understand its mechanism (Gebson *et al.,* 2015; Zhang *et al.,* 2015) and to develop related biomarker for further animal breed (Wang *et al*., 2015). The major fecundity gene they have great effect on prolificacy, litter size and reproductive performance of different goat breeds such as GDF9, BMP15 and also other fecundity gene those has their own effect such as BMPR1B, GH,BMP4, etc. Growth differentiation factor 9 (GDF9), one of the fecundity genes in goats that is found on chromosome 5 is a growth and differentiation factor that is produced by oocytes in mammals and plays a crucial function in early folliculogenesis in female reproduction (Elvin *et al.,* 1999). The caprine GDF9 gene has been investigated as a potential gene for high prolificacy in goats (Ahlawat *et al*., 2015). Mutations in a collection of closely related genes have been shown to considerably enhance the ovulation rate in goats.Farm animals are divided into mono-ovulators (such as cattle and buffalo) and multi-ovulators (such as goat and pig) based on ovulation rate (Hulter *et al*., 2004). Due to its ability to

directly examine genetic diversity at the DNA level, molecular genetics can overcome the limitations of traditional selection methods for the improvement of reproductive traits. Fecundity studies in goats however are difficult to implement in practical situations because reproductive parameters are complicated quantitative traits involving multiple genes, loci and interactions (Ahlawat *et al.,* 2015).

The ovary-derived transforming growth factor (TGF) superfamily includes the bone morphogenetic protein receptor type 1B (BMPR-1B), bone morphogenetic protein 15 (BMP15) and growth differentiation factor 9 (GDF9) (Monsivais *et al.,* 2017). They are commonly referred to as "Fec" genes as a result of their association to fecundity. Initially it was discovered that GDF9 is a growth factor generated from oocytes that is essential to ovarian somatic cell growth. GDF9 causes a decrease in the number of primordial follicles while increasing the number of primary follicles (Vitt *et al.,* 2000)

2.6.1. The association between goat litter size and growth differentiation factor 9 (GDF9)

Goat prolificacy or the average number of kids born per kidding, is a crucial economic trait. Since an increase in offspring size can result in significant increases in profit animal breeders have to pay attention to this traits. By enhancing the genetic potential through appropriate selection techniques, animal geneticists can achieve their persistent goal of increasing profit from livestock. Genetic selection is a key strategy to obtain the best from livestock. Farmers will eventually benefit financially thanks to livestock with greater reproductive capacity and a higher fertility rate (Mishra *et al*., 2017). A crucial and complex economic factor in the goat industry is litter size.(Ray *et al*., 2016) identified that a variety of genes and factors particularly those involved in ovarian follicular development, oocyte maturatio, ovulation, fertilization, embryogenesis, embryo implantation and uterine receptivity appear to cooperatively control litter siz animal breeders since an increase in offspring size can yield substantial gains in profit.

Prolificacy in domestic species genetically influenced by multiple genes called fecundity genes (Gootwine, 2020). BMP15 and GDF9 are two candidate fecundity genes among the several detected in goat so far (Ahlawat *et al.* 2015). From the first stage of follicular development until

ovulation, the growth factor is present in oocytes (Hanrahan *et al.,* 2004). The ovulation rate in animals is increased even further by mutations in the GDF9 gene (Pramod *et al.,* 2013). However, in two out of four mutations follicle development fails in homozygous individuals, resulting in infertility (Nicol *et al*., 2009).

The mutation in the GDF9 gene enhances ovulation rate in heterozygous individuals. An early development stop in follicular growth is the cause of ovarian failure. Goat GDF9gene was significantly more complex and richer than in sheep (Ghoreishi *et al*., 2019). A total of 45 SNP mutations were found in the goat GDF9 gene of which 15 were identified in more than 30 goat breeds worldwide according to the NCBI SNP database (Wang *et al.,* 2019). The same mutation location however it has been shown to have different physiological effects in various investigations. For instance the relationship between a particular SNP locus and reproductive traits varied based on the breed of goat. The same genotype also revealed a positive link with high prolificacy in some goat breeds while a negative correlation with high prolificacy was seen in other goat breeds.

2.7. Measurement of genetic variation at polymorphic loci

2.7.1. Diversity and differentiation: genetic variability measurements

Diversity and differentiation can be studied in population genetics to calculate genetic variation. Differentiation measures variation between two or more populations or subpopulations whereas diversity measures individual variation within a population taking into consideration the number and frequencies of different variants/types (Gregorious, 1987). Several parameters have been developed as diversity estimators using specific models and probabilistic principles. The most often used measure of genetic variation within a population among them is called heterozygosity (H). Natural selection and genetic drift, which have been associated to low genetic diversity and high endogamy, are known to enhance genetic differentiation between populations.In contrast, gene flow increases the homogeneity of genetic diversity within populations leading to little variation and high genetic diversity. Several techniques including Wright's F-statistics have been used to measure the degree of differentiation in population subgroups.(Wright, 1951, 1965)

developed three correlation coefficients that divided the genetic diversity among the entire population (T) and individual people (I).

Population genetic diversity depends on the total genetic variety of the species as well as the percentage of that total genetic diversity that is present among its populations. The most prevalent genetic parameters include the percentage of polymorphic loci (P), mean allele number per locus (A), mean allele number per polymorphic locus (AP), effective allele number (Ae), expected heterozygosity (He), and observed heterozygosity (Ho).When only a small number of loci are taken into account, the parameters P, A, and AP become less useful and less informative.

2.8. Statistics comparison within vs among populations

2.8.1. Heterogeneity/diversity estimators

I.Calculating the effective number of alleles (Ae) using the square root of the sum of allele frequencies

$$Ae = 1/(1-h) = 1/\sum_{i=1}^{n} pi2$$

$$= 1/1-He, 1 < Ae < \infty$$

Where pi is the allele frequency of the i^{th} allele and He is the expected heterozygosity

Ae measures the number of alleles present at a region and assesses how evenly distributed the allele frequencies are there. However it should be noted that populations with various allele frequency distributions may yet have equal levels of Ae.

II.Expected heterozygosity (He), often known as gene diversity, is a composite measure that includes genetic variation at the allele level and measures the likelihood that two randomly selected alleles are different.(Nei, 1978). It is calculated as:

$$H_e = 1-\sum_{i=1}^{n} pik2$$

Where Pi is the frequency of allele i at locus k; n is the number of alleles at locus k

III. For each locus and population, observed genotype frequencies are used to directly calculate observed heterozygosity (Ho). As a comparative measure of diversity, Ho is less informative because it is affected by inbreeding and other evolutionary processes that go against Hardy-Weinberg's equilibrium assumptions.

$$Ho = \sum_{i=1}^{n} ni/N$$

Where ni is the number of heterozygous individuals at the i^{th} locus; N is the total number of individuals analyzed.

The fixation index (FST), which is caused by genetic drift among subpopulations, measures the mean decrease in heterozygosity of a subpopulation (compared to the whole population). It gauges how much subpopulations differ genetically from one another. When there is no differentiation, its value is 0.0, and when there is complete differentiation, or when populations are fixed for various alleles, it is 1.0.

FST = HT-HS/HT, $0 \leq FST \leq 1$ or FST = HT-HS/HT, where 0 = FST and 1 = HS.

2.8.2. Genetic distance and Nei's diversity index

Expected heterozygosity, commonly known as genetic diversity, can be classified into within-population and among-population components. The mean genetic diversity within populations (Hs) and the mean genetic diversity among populations (DST) are added to calculate the total genetic diversity at a locus (HT =1-pi^2, where pi is the mean frequency of the ith allele in the pooled sample) (Nei, 1973, 1978

HT=Hs+DST

GST, a statistic developed by Nei in 1973, was used to measure among-population differentiation in relationship to total diversity:

Nei's genetic distance (D)

The most common approach for calculating the distance between populations or individuals is Nei's (1972) genetic distance. First, for a given locus, the standardized genetic identity (I) is calculated as

$I=Jxy/\sqrt{JxxJyy}$ $0 \leq I \leq 1$

Where Jxx is the probability that two randomly selected alleles from population X have the same genotype, or $Jxx = Pi^2ix$ (sample gene identity in population X); Jyy is the corresponding probability for population Y (sample gene identity in population Y); and Jxy = PixPiy (sample gene identity in both population X and Y). The genetic distance is then calculated by taking the negative of the genetic identity's (I) natural log:

$D = -\ln(I)$, $0 \leq D \leq \infty$

Even while two populations having comparable allele frequencies could have different genotype proportions, they would still have a genetic distance of 0 and a genetic identity of 1. The genetic distance value is assumed to be zero for populations with all homozygotes (F=1) or all heterozygotes (F= -1) respectively.Since GST (and FST) are calculated from expected heterozygosity, which is solely based on allele frequencies, GST in such populations will also be 0.Nei (1978) current D and called it Nei's unbiased genetic distance after accounting for sample size. Given by are the unbiased estimators for JX (J'x) and JY (J'y).

2.8.3. Allelic and Genotypic frequencies

Since evolution is defined as a change in allele frequencies through time and space (Mayr, 1963) allele frequencies are considered as the fundamental genetic traits of a population. Since populations with different genotypic frequencies can have identical allele frequencies genotypic frequencies are significantly less useful. The number of copies of a particular allele divided by the total number of copies of all the alleles at a locus in a population is known as the allele frequency. Allele frequencies are commonly used in population genetics to express the extent of genetic diversity at the individual,population and species level. The sample frequency, pi, of the i^{th} allele is calculated as follows if there are ni copies of the i^{th} allele in the sample population.

pi =ni/2n where n is the total number of sampled individuals

Additional fundamental genetic variables of a population include genotypic frequencies. The number of homozygotes and heterozygotes can be determined by the molecular patterns of DNA bands on a gel, and this information is used to calculate the observed genotypic frequencies. A

particular genotypes frequency can be calculated by dividing the number of individuals possessing that (n) by the entire population sampled (N), or n / N

2.9. Hardy-Weinberg equilibrium/principle

Important to compare allele frequencies in agiven population over period of time Hardy-Weinberg equilibrium. In the absence of disruptive factors like nonrandom mating, mutation, selection, a limited population size, overlapping generations, random genetic drift and gene flow, it states that both allele and genotype frequencies in a population remain constant or are in equilibrium from one generation to the next. However Hardy-Weinberg equilibrium.never occur in nature because there is always at least one above rule being violated. Thus, the idea of genetic equilibrium is an ideal state that offers an initial basis to measure genetic change in populations across generations. Based on a simple binomial (for two alleles) or multinomial (for a number alleles) distribution of the allele frequencies, this equilibrium can be mathematically represented.

The standard definition for the simplest situation of a single locus with two alleles includes a locus designated A with alleles A1 and A2. Alleles A1 and A2's respective frequencies are represented as p1 and p2, respectively, with (p1+p2 =1). The frequencies and proportions of the different genotypes in a population that is in Hardy-Weinberg equilibrium are as follows. A1A1: p. 12, A1A2: p.1 and 2, and A2A2:p. The expected HWP are pi2 for homozygotes (AiAi) and 2pipj for heterozygotes (AiAj) for a locus with k distinctive alleles and allele frequencies pi (pi =1), where ij and i, j =1, 2,..., k.When applied to population level variation, Mendel's first law of independent segregation of the two parental alleles provides these predictions.

3. MATERIALS AND METHODS

3.1. Description of the study area

The Morphological data and blood samples collection were performed in Drra district which is one of the districts in North Shoa Zone Oromia Regional State and located 221km from capital city, Addis Ababa. and 110 km from zonal town of North Shoa Zone (Fiche) (figure 1). It is bordered on the south by the Jama River which separates it from Hidhabu Abote, and Warajarso on the West, north and east by the Amhara region the Abay River which separate it from Shebal Berenta, on the East by Wenchit stream which separate it from Mida Oromo and Merehabete district, on the North by Weleka Stream which separate it from Wogidi and kelala district of Amhara Regional State. The latitude of Dera is 10.1667 ($10^0$09, 60.3.9600"N and longitude is 38.6667($38^0$39, 59.99"E) while its elevation range from 700 –2575 masl which contains highland 20%, midland 30% and lowland 50%. The average daily temperature ranges from 18°C to 25°C, while the annual rainfall ranges from 800 to 1000 mm and occurs in a bimodal pattern with minor rains in March and April and major rains in July and September. The total land area of the district is about 159681 hec. Out of these, cultivated land accounts 55%, grazing 15%, natural forest 16.97%, plantation forest 8.5%, Shrubs 4.53% (Derra district rural land use management office, 2019). The total Livestock population is about 439,351 from these 27.62 % are goat (Derra district livestock and Fishery Development office statics, 2019).

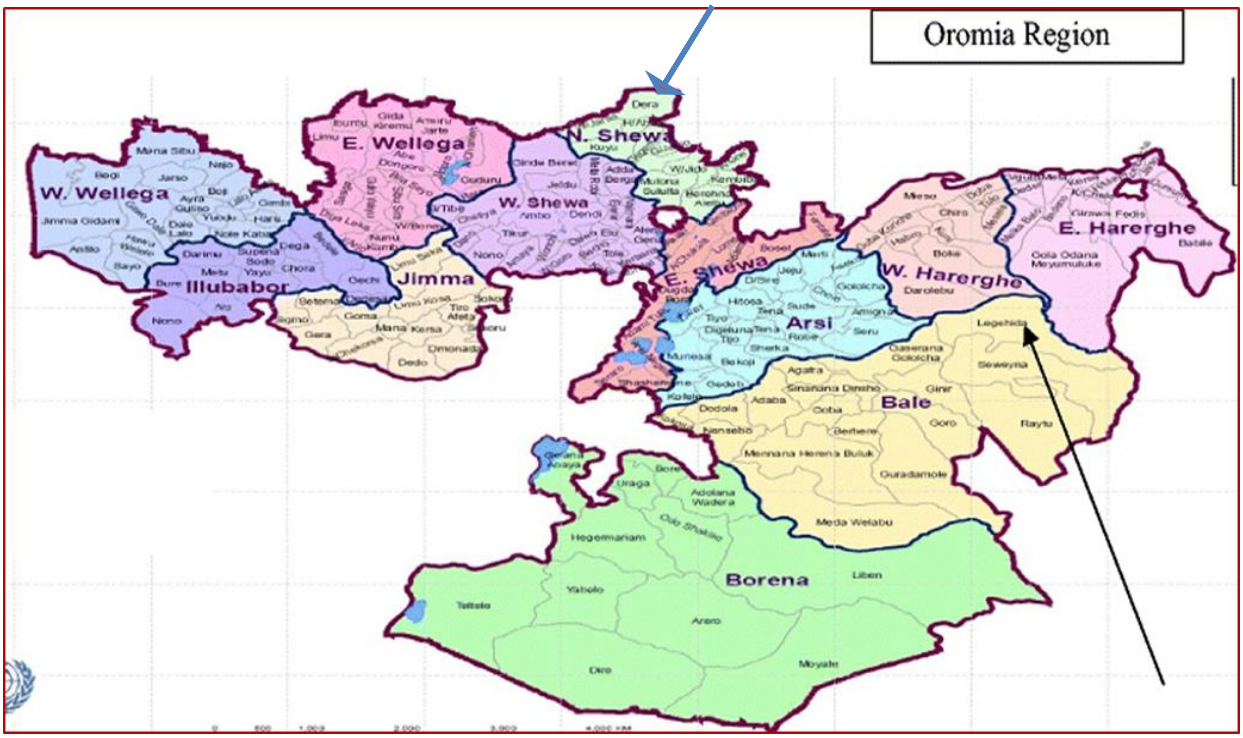

Figure 1. Map of the study

3.2. Sampling procedure and data collection

3.2.1. Morphological sample collection

Sampling site Derra district, was selected purposively based on its potential for goat production and availability of goats with high prolificacy. Information was gathered from discussion together with district professionals and experts. Stratification sampling method was done based on agro ecology (highland and lowland) and litter size seven keble's having high goat population with twining, triplet and single litter size were selected purposively for morphological data. A total of 336 individual were sampled for the study from these 282 were female goats and the 54 was male goat (Table 4). To study inheritance trait of fecundity gene however the number of data collected for male and female was not equal/standard to analyisis due to these reason male sample was not used for association in the study. The qualitative character coat color pattern, coat color type and hair type. The quantitative character also such as Body weight, Chest girth , Body length, Wither height, Rump height, Chest depth, Pelvic width, Pelvic length, Tail length.

Table 4. Morphological data collected from local goats with different birth type in the study areas

Litter size	Morphological sample	Sampling site	Agro ecology
Singleton	30	Gundomeskel	Highland
Twin	43	Addisalemyaya	Highland
	41	Degnuwebenso	Lowland
	42	Qoro	Lowland
Triplet	43	Kabigolelcha	Highland
	42	Adeamelke	Highland
	41	Harbodeso	Lowland
Total	282	7 Kebles	3 Lowland and 4 Highland

3.2.2. Blood sample collection

Blood samples were collected from only 150 does considred for morphologica data collection (Table 5). Information on parity was also collected during blood sample collection. Blood samples (approximately 4ml) were collected by professional veterinarian and transported refrigerated by ice box to Addis Abeba University's Genetics Laboratory, Department of Microbial and Molecular Biology. DNA extraction and further analysis also conducted at Genetics Laboratory Department of Microbial and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia. Only female goats (does) (282 samples) were considred due to limited number of mature and small number male goat for this study because the male goat sample used only to check inheritance trait/character of growth differentiation factor 9 gene(GDF9gene) through generation however the best involved sample to study fecundity is female goat since fecundity highly related with female goat than male goat.

Table 5. Blood samples data collected from local goats with different birth type in the study areas

Litter size	No of blood samples	Sampling site	Agro ecology

Singleton	30	Gundomeskel	Highland
Twin	20	Addisalemyaya	Highland
	20	Degnuwebenso	Lowland
	20	Qoro	Lowland
Triplet	20	Kabigolelcha	Highland
	20	Adeamelke	Highland
	20	Harbodeso	Lowland
Total	150	7 Kebles	3Lowland and 4 High land

3.3. DNA extraction and Polymerase chain reaction (PCR)

DNA extraction and quality assessment were done at Addis Ababa University, Molecular Biology Laboratory. The salting out procedure was used for extracting the genomic DNA from whole blood (Bruford *et al.,* 1992). Blood samples were thawed to room temperature and 1.5 ml of blood was mixed with 4.5ml of cold EL buffer (0.155M NH4Cl, 10mMNaCO3 and 1mM EDTA, pH 7.4) in a falcon tube and placed on ice for 15 minutes while mixing occasionally. The supernatant was removed from the tube after it had been centrifuged for 10 minutes at 800 g (3500 rpm) while still retaining the pellet. The pellet was then re-suspended in 3 ml of lysis buffer (KL buffer:10 mM Tris, 2 mM EDTA, 0.4 M NaCl, pH 8.2) after being washed twice with EL buffer until there was no sign of hemoglobin remained. The resulting cell lysates were treated overnight at 370°C with 150 µl of 20% SDS and 100 µl of proteinase K (20 mg/ml), with gentle shaking. After the completion of the process of digestion, 1.5 ml of saturated NaCl (6M) total volume was added to each tube and shaken vigorously for 15 seconds. The tubes were then centrifuged at a temperature-controlled 1700 g for 15 minutes. After retaining the precipitated protein pellet at the bottom of the tube, 600 µl of the supernatant containing the DNA was transferred to a 2 ml microcentrifuge tube. When the DNA was starting to precipitate, two volumes (1200 µl) of absolute ethanol kept at room temperature were added. The tubes were then continuously inverted.

The precipitated DNA was then spooled out using a plastic pipette, washed several times in 70% ethanol, and then inserted in a 1.5 ml microcentrifuge tube containing 0.5 ml TE buffer [10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4]. The DNA was after that left to degrade at 4°C for a full night

(Bruford et al., 1992). By using a UV spectrophotometer to determine the purity and quantity of genomic DNA, optical density (OD) values at 260 and 280 nm were recorded with TE buffer serving as a blank.By using the OD(260/280) value, the concentration of genomic DNA was calculated spectroscopically. Samples with an acceptable optical density ratio (OD260/OD280) of 1.8 to 2.0 were selected for the polymerase chain reaction.By performing agarose gel electrophoresis and viewing the results with a gel documentation system (BIO-RAD (Gel DocTMEZ imager), the quality of an isolated genomic DNA sample was evaluated. Genomic DNA concentrations were adjusted to 50ng and ranged from 100ng to 25ng. A pair of primers, F:GAAGACTGGTATGGGGAAATG and R:CCAATCTGCTCCTACACACCT which were adopted from (Noshahr and Rafat, 2014), were used to amplify the GDF9 gene exon 1.

The final volume of the polymerase chain reactions was 20 µl, which contained 1 µl of genomic DNA (50ng), 0.5 µl of each primer (10 pmole), 4 µl of master mix (dNTP, Taq polymerase, PCR buffer and $Mgcl_2$)and 14 µl of nuclease-free water. The GenAmp® PCR System 9700 (Applied Biosystems) was used for touchdown PCR under the following conditions:Following a three-minute initial denaturation at 95°C, there were 35 cycles of 30s ea ch at 95°C, 30s at annealing temperatures (between 65°C and 70°C), and 30s at 72°C. After the reactions had been completed, a final extension was conducted at 72°C for 5 minutes by using gel electrophoresis (2 percent agarose gel) the PCR results were examined. A 1kb plus DNA ladder in the gel was used to measure the allele size of the GDF9 gene, and a UV transilluminator was used to see the results

3.4. Data Analysis

3.4.1. Morphological Data Analysis

All data gathered during the study period were coded and recorded. The statistical package for social sciences (SPSS, 23) was used to analyze observations on qualitative traits separately for female goats. The Chi-square (x^2) test, Pearson correlation analysis of SAS version 9.0 were also used to test the statistical significance of categorical variables. Depending on the type of data, different statistical analysis techniques were applied (SPSS, 2023) performed the Pearson correlation test to determine the correlation between body weight, linear body measurements, litter

size and GLM analysis. Categorical variables were generalized and described using descriptive statistics.

3.4.2. Genetic Variation / polymorphism Analysis

All 150 samples were subjected to PCR analysis but only 118 samples were amplified and allele and genotype were scored. Allele frequency for GDF9 gene was determined by Image lab 6.1software using 1kb plus ladder. The GDF9 locus' allele frequencies, observed and expected genotype frequencies, and chi-square test were calculated using PopG ene 1.31 (Yeh *et al.*, 1999). Using PopGene 1.31 (Yeh *et al.*, 1999), the observed (HO) and expected (HE) heterozygosity, effective number of alleles (Ne), and proportion of polymorphic loci were calculated.

3.4.3. Association Analysis

The linear model with fixed effects was used to analyze the relationships between genotypes GDF9 gene and litter size using the following model: $Y_{ij}= \mu+M_i + P_{j+} e_{ij,}$ where Yij is the litter size's phenotypic value and μ is the population mean. Pj is the fixed effect for the j^{th} parity, eij is the random error effect of each observation, and Mi is the fixed effect of the i^{th} genotype. The correlation test between body weight, linear body measurements with litter size and the GLM analysis was performed by SPSS version 22 (SPSS, 2013).

4. RESULTS

4.1. Morphological variation among goat gopulation

4.1.1. Qualitative and quantitative traits of the sampled goat population

Coat color Pattern

The qualitative triat of indigenous goats found in Darra districts are shown in Table 6 and Figure 2. A chi-square test revealed a significant difference ($P<0.05$) between goat populations across different locations in terms of coat color pattern. The most common coat color pattern observed in the study area was plain71% goat population of degnu, 70% gumesqel, 66%, kabi,62% addis alemyaya, 52.5% adeamelke, 51% qoro and 43% harbodeso.

Coat Color type

The qualitative triat of indigenous goats found in Darra districts are shown in Table 6 and Figure 2. A chi-square test revealed non-significant difference ($P>0.05$) between goat populations across different locations in terms coat color type these result indicate coat color type has no significant effect to select the most prolific goat in the study areas. Spotted, red and brown respectively were observed and in the sample populations brown with different colors (red, black ,white), uniform red, black, white, coat color types were also observed with small and varied frequencies (Table 6). This indicated that goat populations found in the study area had a wide range of coat color types. Black coat color was less frequent than white and other.coat color pattern and hair type showed.

Hair type

The qualitative triat of indigenous goats found in Darra districts are shown in Table 6 and Figure 2. A chi-square test revealed a significant difference ($P<0.05$) between goat populations across different locations in terms of hair type. The most frequent observed hair type Straight hair, glossy and smooth hair respectively in different location. Location significantly affectect hair type of goat population in the study areas it implicate how farmer select productive goat bytheir hair type.

Table 6 Mean observed qualitative characteristics of female in the study areas.

The effect of location on qualitative trait										
Morphology	Trait	AY	AM	KG	DW	HD	QO	GU	X^2	Pvalue
Coat color pattern	P	2(5)	9(22.5)	5(6.8)	2(5)	6(14)	11(28)	5(16.7)	26.82[a]	.010**
	PL	25(62	21(52.5)	29(66)	28(71)	18(43)	20(51)	21(70)		
	Sp	13(32)	10(25)	12(27)	9(23)(	18(43)	8(20.5)	4(13)		
Hair type	SH	21(55.5)	26(65)	19(43)	20(51)	20(47.6)	19(49)	18(60)	29.105[a]	.004**
	SS	15(12.5)	6(15)	17(36.8)	2(5)	13(31)	5(13)	2(16.7)		
	G	14(35)	8(20)	8(18)	17(43.6)	9(21)	15(38.5)	7(23)		
Coat color type	BL	2(5)	3(7.5)	3(6.8)	6(15)	2(5)	1(2.6)	3(10)	41.284[a]	.082
	BR	7(17.5)	7(17.5)	16(36)	6(17)	5(12)	5(13)	9(30)		
	P	2(5)	4(10)	3(6.8)	0(0)	3(7.5)	0(0)	3(10)		
	R	15(37.5)	11(27)	9(20.5)	11(28)	8(19)	11(28)	5(16.7)		
	SP	13(32.5)	13(32)	11(25)	11(28)	21(50)	18(46)	6(20)		
	W	1(2.5	2(5)	2(4.5)	5(13)	3(7)	4(10)	4(13.5		

Note: PL-Plain ;P-Patchy ; Sp-Spotted ;G-glossy; SS smooth hair ;SH-straight hair ;W-White ; BL-black ;Br- Brown ; P-different color ;R-red ;SP -spoted ;F-female;M-male ;AY-Addisalemyay ;AM-Adeamelke ;KG-Kabigolcha ; DW-Degnuwebenso ;HD-Harbodeso ;QO-Qoro ;GU-Gu/mesqel

4.1.2.The effect Climatic/Agroecology factors on qualitative trait of goat in the sudy areas

Agroecology effect:

Coat colar type significantly affected by agroecology at ($p<0.05$) so it help to select which goat coat color type adapted in lowland and highland agroecology to ensure their surviaval and reproduction ability of goat how ever agroeclogy has no significant effect on coat color pattern and hair type at ($p>0.05$) on goat population in the study areas.

Table 7 Mean observed for qualitative traits affected by agroecology

Morphology	Trait		Highland	Lowland	X^2	Pvalue
Coat pattern	Patchy		9(12)	19(16)	1.577a	0.455ns
	Plain		96(59)	66(55)		
	Spotted		39(25)	35(29)		
Hair type	Straight hair		84(54.5)	59(49)	3.601a	0.165ns
	Smooth hair		33(21)	20(16.7)		
	Glossy		37(24)	41(34)		
Coat color type	Black		11(7)	9(7.5)	13.59a	0.018**
	Brown		39(25)	16(13)		
	Plain		12(7.8)	3(2.5)		
	Red		40(26)	30(25)		
	Spotted		43(28)	50(41.7)		
	White		9(5.8)	12(10)		

Note: p>0.05 ns =non-significant

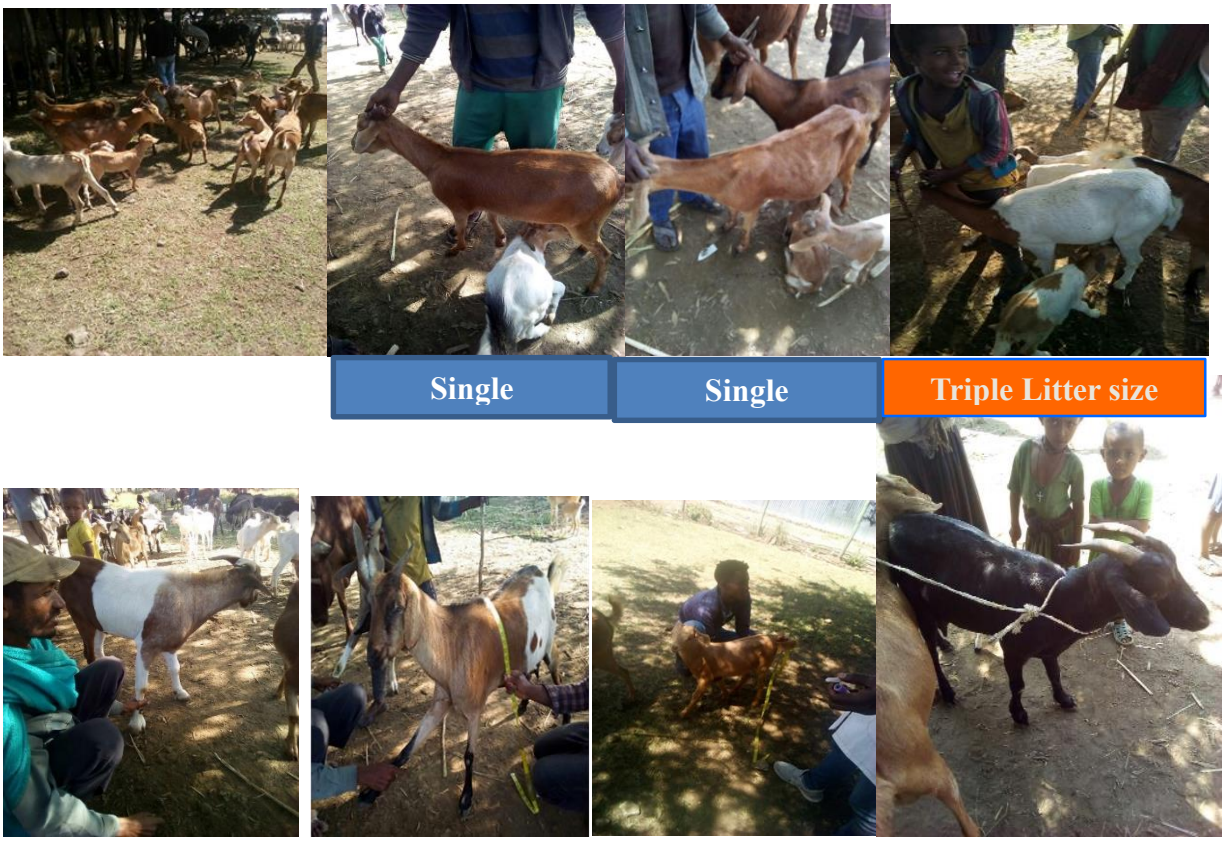

Figure 2. Representative picture of female, male and number of litter goats in derra district /study areas

4.1.2. Pearson Correlation of the Quantitative Traits

In derra goat population result on below table 8 show that body weight and most lenear body measurement have highly significant and strongest positive correlation at ($p < 0.001$) between each lenear body measurement since changes in body weight have an important effect on linear body measurements, those measurements are more essential to estimating an animal's live body weight. However, tail length, pelvic length and pelvic width have positive small correlation between linear body measurement this result indicate each quantitative trait have their own effect on the prolificacy /litter size of goat population. The strongest positive and highly significant relationship between BW and CG and BL indicated that CG may be able to provide the best estimate in predicting live BW of goats, however the correlation between tail length and body weight and body length is not very strong.in the fields of study.

Table 8 Pearson's correlation coefficient values among morphological traits of local goats

Variable	Body Weight	Body length	Wither Height	Chest depth	Chest girth	Tail length	Pelvic width	Pelvic length	Rump height
Body Weight	XXX								
Body length	0.57***	XXX							
Wither Height	0.56***	0.59***	XXX						
Chest depth	0.99***	0.57***	0.55***	XXX					
Chest girth	0.53***	0.456**	0.41*	0.536***	XXX				
Tail length	0.216*	0.207*	0.305*	0.215*	0.233*	XXX			
Pelvic width	0.287*	0.275*	0.299*	0.291*	0.415**	0.290*	XXX		
Pelvic length	0.437**	0.472**	0.409**	0.438**	0.392*	0.129*	0.122*	XXX	
Rump height	0.437**	0.467**	0.58***	0.439**	0.38*	0.19*	0.24*	0.407**	XXX

Note* $p \leq 0.0\,5$; * * * $p \leq 0.0\,0\,1$; C G, chest girth c; B L, body length ; W H, wither height ; R H, rump height C D, chest depth P W, pelvic width PW,pelvc length ;TL,tal length(below diagonal has Low, medium and high positive correlation between LBM)

4.1.3. The Effect of Agroecology on Body weight and Linear body measurements

The body weight and linear measurements for the studys areas with different effects are shown in Tables 9 and 10.

Agroecology effect: The least square mean for all varialbles showed highly signicant ($p < 0.001$) difference between agroecologies i.e highland and lowland. Highland goats showed better body weight and liner body measurments expet rump height, Tail length, Pelvic length compared to the lowland goats.

Table 9 Least-squares means and standard error for body quantitative traits affected by agro ecology

Fixed variable	No	BW LSM±SE	BL LSM±SE	WH LSM±SE	CD LSM±SE	CG LSM±SE	TL LSM±SE	PW LSM±SE	PL LSM±SE	RH LSM±SE
Over all		28.2	61.97	62.7	13.6	69.40	12.2	13.23	15.86	65.3
CV%		12.3	7.69	6.81	13.65	7.75	12.29	12.95	12.45	5.94
Agroecology	282	***	***	***	***	***	***	***	***	***
Highland	157	29±0.3	62±0.36	63±0.3	14±0.1	70.5±0.4	12±0.1	14±0.1	16±0.16	65±0.3
Lowland	125	27±0.3	62±0.4	62±0.4	13±0.16	68±0.5	12±0.1	12.5±0.1	16±0.16	65±0.3

Note*** $p \leq 0.0\,0\,1$; BW; body weight C G, chest girth c; B L, body length ; W H, wither height ; R H, rump height ; C D, chest depth; P W, pelvic width ; PW,pelvc length ;TL,tal length LSM=least mean square, SE=standard error, CV= Coefficient of variation.

Location effect:The least square means for all variables were highly significant ($p < 0.001$) across different location (Table 10). Body weight and linear body measurement has highly significantly affect the goat in Addisalem yaya and Kabigolcha than other location.

Table 10 Least-squares means and standard error for body quantitative traits affected by Location

Fixed. Variable	No	BW LSM±SE	BL LSM±SE	WH LSM±SE	CD LSM±SE	CG LSM±SE	TL LSM±SE	PW LSM±SE	PL LSM±SE	RH LSM±SE
Overall		28.2	62.0	62.7	13.6	69	12.2	13.2	15.8	65.3
CV%		12.2	7.55	6.7	13.3	7.7	11.4	11	11.8	5.87
Location	282	***	**	***	***	**	**	***	***	*
AY	40	29^{c}±0.6	60^{ab}±0.8	64^{bc}±0.6	14^{bcd}±0.3	71^{bc}±0.9	12^{ab}±0.2	16^{bc}±0.1	13^{ab}±017	64^{a}±0.6
DW	42	27.6^{b}±0.5	63^{b}±0.8	63^{abc}±0.8	14^{bc}±0.3	68^{ab}±0.8	12^{ab}±0.1	17^{c}±0.3	12^{a}±0.2	65^{ab}±0.7
GU	30	27^{a}±0.6	61^{ab}±0.8	62^{ab}±0.8	15^{d}±0.3	68^{a}±0.9	13^{b}±0.2	14^{a}±0.2	16^{c}±0.25	65^{ab}±0.7
HD	42	27^{a}±0.5	61^{ab}±0.8	61^{a}±0.6	12.6±0.3	68^{ab}±0.8	12^{a}±0.2	15^{b}±0.3	12.5^{a}±0.3	70^{ab}±0.5
KG	45	29^{c}±0.4	63^{b}±0.9	64^{c}±0.9	14^{cd}±0.2	71^{c}±0.9	14^{c}±0.3	16^{c}±0.3	13.5^{b}±0.3	67^{b}±0.6
QO	41	27^{a}±0.6	59^{a}±0.9	61^{a}±0.7	13^{a}±0.3	67^{a}±1.0	12^{ab}±0.2	15^{b}±0.3	12^{a}±0.2	65^{a}±0.6
AM	42	29^{c}±0.4	62^{ab}±0.6	62.5^{ab}±0.5	13^{ab}±0.3	71^{c}±0.7	12^{a}±0.2	16^{bc}±.02	13^{ab}±0.2	65^{a}±0.4

Note*** $p \leq 0.0\ 0\ 1$ BW; body weight C G, chest girth c; B L, body length ; W H, wither height ; R H, rump height ; C D, chest depth, P W, pelvic width,PW,pelvc length ;TL,tal length LSM=least mean square, SE=standard error, CV= Coefficient of variation

4.2. Genetic variation and association of GDF9 gene with Litter size

4.2.1. Morphological variation and its association with prolificacy/ litter size

The analysis of variance of the morphological traits in local/ indigenous goats with different litter size is shown in Table.11. Mean comparisons among does with different litter size showed differences ($p<0.05$) for all traits considered in the study. Most of the height (withers and rump height), conformation (chest girth and chest width), and body weight and body length were higher in does having twin and triplets. However, there was no variation in most of measurements except pelvic length among does having twin and triplets (Table 11) and(Figure 2). The majority of linear body measurements in the study area's goat population indicated an association with litter size. The body weight, body length, and chest girth of a goat with twins and triplets are higher than those of a goat with a single birth or a small litter.

Table 11 Morphological variation among local goats with different litter size (N=150)

	Litter size				
Parameters	Singleton(n=30)	Twin(n=60)	Triplet(n=60)	SEM	*P*
Body weight	26.05^{b}	27.79^{a}	28.19^{a}	0.43	0.00
Body length	55.92^{c}	61.77^{b}	64.99^{a}	0.41	0.00
Whither height	59.46^{c}	62.49^{b}	64.80^{a}	0.38	0.00
Rump height	62.88^{c}	65.61^{a}	67.04^{a}	0.42	0.09
Chest girth	65.13^{b}	69.31^{a}	70.46^{a}	0.57	0.00
Chest width	12.76^{c}	13.59^{b}	14.71^{a}	0.16	0.07
Pelvic width	11.98^{b}	13.08^{a}	13.32^{a}	0.15	0.00
Pelvic length	14.84^{c}	16.13^{b}	16.85^{a}	0.29	0.00

abc Means in the same row without common letter are different at $p<0.05$; SEM,Standard error of the mean

Amplified GDF9 gene at exon 1 region

Figure 3 shows genomic DNA was amplified using a pair of primers that covered exon 1 sequence of GDF9 gene. Electrophotograph of the 462 and 478bp GDF9 fragments generated from PCR amplification of genomic DNA using GDF9 specific primers can be seen in the electrophotograph. A 1 kb plus bp DNA ladder is located in lane L.PCR result of the local goats' exon 1 GDF9 gene with identified allele size and their frequency is shown in Figure 3 and 4. Two alleles 462 and 478 and three genotypes, 462/462; 462/478 and 478/478 were identified in local goats with different

litter size. Allele 462 with different frequency was observed in most goat samples considered, however, allele 478 was identified only in does having twins and triplet kids. The observed result indicate the exsistance two fragment so it indicate there is genetic variation among and within goat population in the study areas in some instance it implicate exsistance of polymorphism.

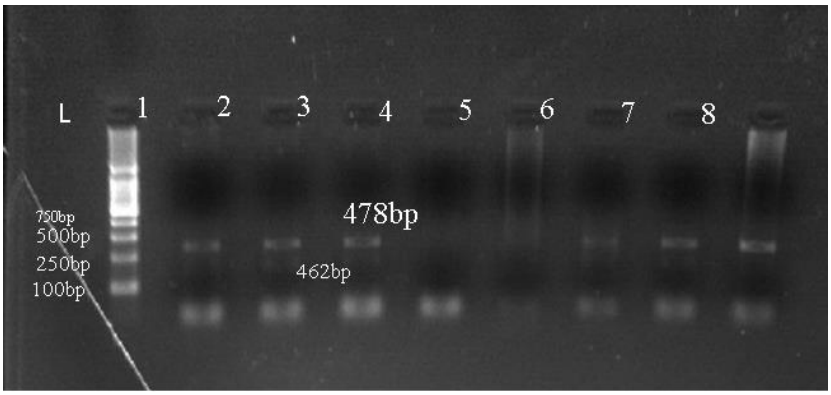

Figure 3. PCR result obtained from the GDF9 gene's exon 1 with different allele size in Ethiopian local goats

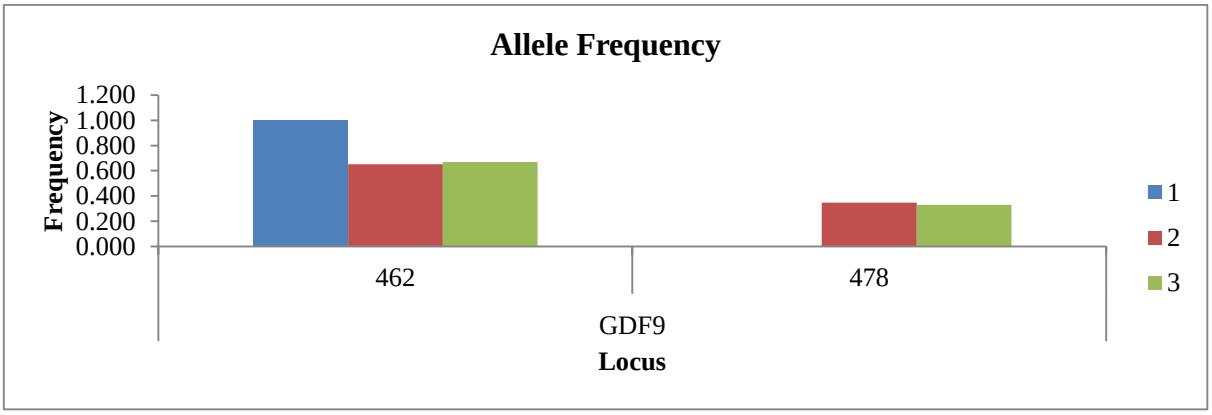

Figure 4. Allele frequency of GDF locus in local goats with different birth type

4.2.2. Allele and genotype frequency at the GD9 locus

Allele and genotype frequency of GDF9 gene in local goats are given in Table.12 below. GDF9 gene showed three genotype (462/462, 462/478 and 478/478) in local goats with different litter size, but segregating with different frequencies. Allele 462 was fixed in does with single litter size and only homozygous genotype, 462/462 was observed in the samples. However, the same allele was observed with different frequencies 0.65 and 0.67 in does with twins and triplets litter size,respectively. Allele 478 was observed in both does having twins and triplet litter size with relatively similar frequency.Genotype 462/462 was more predominant in does with twin compared to triplet, however, 462/478 genotype relatively higher in does with triplet than twin. No variation

(p>0.05) of GDF9 allele frequencies was observed among the local goats which might indicated that local goats were under Hardy-Weinberg equilibrium for GDF9 locus.

Table 12 Allele and genotype frequency of GDF9 gene in local goats with different birth type

Litter size	Allele	Allele frequency	Genotype	Genotype frequency	Genotype observed	Genotype expected	χ^2 df=2
Singleton	462	1.00	462/462	1.00	6	6	Monomrphic
	478	0.00	462/478	0.00	0	0	
			478/478	0.00	0	0	
Twin	462	0.65	462/462	0.47	24	23.79	
	478	0.35	462/478	0.43	25	25.42	0.90^{ns}
			478/478	0.10	7	6.79	
Triplet	462	0.67	462/462	0.38	23	25.11	
	478	0.33	462/478	0.47	29	24.78	0.20^{ns}
			478/478	0.15	4	6.11	

ns=not significant at (p<0.05)

4.2.3. Genetic variation within Local goats at GDF9 locus

The levels of genetic variation within population are given in Table 13 below.GDF9 locus was monomorphic in goats have singleton but it found to be polymorphic in goats having twin and triplet. Expected heterozygosity (H_E) and the observed heterozygosity (H_O) estimates within goats having twin litter size were similar.However, the H_O was higher than the H_E in goats having triplet litter size. A positive correlation was observed between the effective numbers of alleles per locus and mean H_E.

Table 13 Population genetic indexes at GDF9 exon 1 region among local goats with different birth type

Parameters	Birth type			Over all
	Singleton	Twin	Triplet	
Effective allele no (Ne)	1	1.83	1.79	1.54
Percent of polymorphic loci	0	100	100	66.7
Observed Homozygosity(H_o)	0.00	0.45	0.52	0.33

Expected Hetozygosity (H_E)	0.00	0.45	0.44	0.30

Nei's (1972) standard genetic distances and identity between local goats with different litter size are shown in Table 14 below. Standard genetic distance between litter size pairs was estimated in order to assess the presence of genetic similarity and dissimilarity among goats with different litter size. The shortest genetic distance between twin and triplet was quite low while the largest genetic distance value was found between singleton and triplet though there was substantial distance was observed between singleton and twin

Table 14 Measures of genetic distance/similarities among local goats with different birth litter size

Litter size	Singleton	Twin	Triplet
Singleton	***	0.91	0.84
Twin	0.09	****	0.98
Triplet	0.16	0.02	***

Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

Analysis molecular variance (AMOVA) for local goat with different litter siz at GDF9 locus are presented in Table.15 below. The AMOVA results showed that 52% of the genetic variation of goat population was observed within individual having different litter size and 27% of variation is among population of different litter size. High genetic variation was observed within goat population having diffrent litter size in the study areas.

Table 15 Results of analysis molecular variance (AMOVA) of goat population with different litter size at GDF9 locus

Source of variation	Df	SS	MS	Est.var	P%	NM	Pvalue
Among populations	2	17.04	0.52	0.09	27%	0.69	0.0001***
Among Individual	147	48	0.32	0.07	22%		
Within individual	150	26.5	0.177	0.177	52%		
Total	299	91.3		0.34			

4.2.4. Association of GDF9 genotype and Litter size in Local goats

Least square mean of the litter size for the genotypes of GDF9 gene in local goats are presented in Table.16 below. The general linear model analysis showed that GDF9 genotypes have significant effects ($p<0.05$) on litter size of local goats both at the first and second parity. All three, 462/462, 478/478 and 462/478 observed genotypes has positive effect on litter size in both parity 1 and 2. However, genotype 478/478 was associated with higher litter size compared to the rest of the genotypes.

Table 16 Association GDf9 genotypes with litter size in different parities

		Litter size			
Genotypes	**N**	**Parity 1**	**Parity 2**	**SEM**	***P***
462/462	57	2.16^{b}	2.26^{b}	0.12	0.00
462/478	50	2.48^{bc}	2.62^{bc}	0.07	0.00
478/478	11	2.80^{c}	2.82^{c}	0.09	0.00

abc Means in the same row without common letter are different at $p<0.05$; SEM = Standard error of the mean

4.2.5. Association of GDF9 genotypes with body measuremts of local goats

Association of GDF9 genotypes with body weights and linear measurements of local goats is shown in Table 17 below. Mean comparisons among goats with different genotypes showed significant differences ($p<0.05$) for all traits considered except pelvic length. Most of the height (withers and rump), conformation (chest girth and chest width) and body weight and body length were higher in genotypes associated with higher litter size.However, there was no difference ($p>0.05$) in most measurement between genotype 462/478 and 462/462 except whither height and chest girth.

Table 17 Association of genotypes with body weight and linear body measurements in goats

Parameters	478/478 (N=11)	462/478(N=54)	462/462(N=47)	SEM	*P*
Body weight	27.65^{a}	23.54^{b}	23.41^{b}	0.49	0.04
Body Length	66.18^{a}	63.00^{b}	62.21^{b}	0.47	0.02
Whither height	66.36^{a}	64.08^{b}	62.25^{c}	0.43	0.03
Chest width	14.82^{a}	13.32^{b}	14.30^{ab}	0.19	0.01

Chest girth	75.00[a]	70.34 [b]	70.47 [b]	0.54	0.00
Pelvic width	18.11[a]	16.40[b]	16.37[b]	0.14	0.00
Pelvic length	13.78[a]	13.25[a]	12.94[b]	0.22	0.00
Rump height	68.91[a]	65.78[b]	65.49[b]	0.52	0.04

abc Means in the same row without common letter are different at $p<0.05$; SEM = Standard error of the mean.

5. DISSCUSSION

5.1. Morphological variatation

5.1.1. Qualitative traits

Information on morphological traits is required for long-term breed improvement, genetic resources utilization and conservation. Both quantitative and qualitative morphological raits can be utilized to describe a breed's or population's characteristics. Different genetic, environmental, and rearing management can have an influence on these traits (Sumantri *et al.*, 2007; Wibowo *et al.*, 2021). The plain coat color pattern was the most frequent one observed in the study area and was additionally observed in the North Shewa Zone, Amhara Region, Ethiopia (61.0% in Berehet, 69.0% in Basona-worena, and 76.7% in Minjar-shenkora(Hailu Tilahun, 2019). Similar to this, (Ahmed seid, 2013) found that in the Horro Guduru Wollega zone, 75.49% of the goat population had a simple coat color pattern.According to Kefyalew Alemayehu, (2020), 66.28% of Arab goats were plain. However, a patch coat color pattern was found in half (44.5%) of the goat population in the West Gojam zone (Bekalu Muluneh, 2014).

In the Darra district, spotted, red and brown, and brown with various colors (red, black and white) were the most prevalent coat color types. Additionally, uniform red, black, and white coat color were observed in a variety of small and varied numbers. This indicated that the goat populations found in the study area had a wide variety of coat color types which is in line with the results of (FARM Africa, 1996), who reported that the most common coat color type were spotted (12.67%) brown/fawn coat types in the Bahirdar Zuria, Yilmana Densa and Gonji Kolela districts. On the other hand, the Afar region reported a red-dominant coat color, while the Babbile district reported a white-dominant coat color (42.5%) (Mahilet Dawit, 2012). The Central Highland goat's dominant coat color type was reddish-brown.For the description of Central Highland goats, (FARM Africa, 1996) covered a very large area (central Tigray, Wollo, Gondar and Shewa), and there might be heterogeneity. Goats in Tanqua Abergele (TA)and Kola Tembien (KT) districts had predominantly red and red with white in dominant patterns of plain and patchy a combination of straight and curved horn shape. While goats in Adwa district were showed predominantly red, red with black, red with white and grey in the pattern of patchy and plain, and straight horn shape (Birhaniea.M *et a*l., 2019)

The number of black coats was less compared to that of white and red coats. This indicates that farmers have a preference for a particular coat color. Coat colors of indigenous goat populations are influenced by a number of factors, including genetic background, diversity, environmental factors, and human selection. The most frequent observed hair type Straight hair, glossy and smooth hair respectively in different location. The majority of goat population in Berehet (79.5%), in Basona-worena, (67.6%) and in Minjarshenkora (69.5) had smooth hair coat type and small proportion of them had long straight hair (12.7), glossy (4.0%) and dull (4.8%) haired coat types (Hailu Tilahun, 2019).

5.1.2. Quantative traits

The strongest positive and highly signicant correlation between body weight and chest girth and body length suggested that chest girth could provide the best estimate in predicting live BW of goats however tail length and bodyweight, body length has small positive correlation.in the study areas. Body weight had positive and highly significant ($p< 0.001$) associations with all linear body measurements in both Arab and Oromo in north western Ethiopia goat populations (Kefyalew Alemayehu, 2020). Body weight and other physical characteristics of several Ethiopian goat breeds. From these quantitative measurements, the most strong relationship was found between heart girth and body weight for both sexes ($r= 0.92$ for males; $r= 0.91$ for females). This indicated that either heart girth only or by combining with other quantitative traits could suggest a good estimator for predicting live body weight for the goats((Hailu Tilahun, 2019).Similarly Bekalu Muluneh, 2014), Alubel Alemu, 2015), Alefe Takele, 2015) and (Tsigabu Gebreslassie, 2015) on indigenous goats reported that highest correlation between body weight and chest girth. This shows that heart girth might be the best trait to estimate live body weight for goats and other livestock species. The significant positive and significant association between body weight and heart girth observed in the study provides strong support for the findings of (Girum Gebreyesus *et al.,* 2013) and (Mahilet Dawit, 2012).

Linear udder measurements of sampled goat populations of Central Zone of Tigray, Ethiopia were strongly positively correlated with most linear body measurements, except with body length, rump length, and height at pelvic (Birhaniea *et al*., 2019). Most of linear measurements in the goat population in the study's area indicated correlation with litter size. A goat with twins or triplets will have a larger body weight, length, and chest girth than a goat with a single birth or a small litter.Goats that were native to Cameroon had the same results (Kouam *et al.,* 2015). There was a

significant variation in body length, whither height, chest width, and pelvis length across the three litter size categories. Indian and Cameroon goats had significant positive associations between litter size and body length, withers height, and chest girth (Haldar *et al,* 2014; Kouam *et al*, 2015. These results are supported by the facts.On the other hand(Mule *et al*., 2014) showed that an increase in body weight followed by a rise in chest circumference would simultaneously boost uterus ability to support an increase in the number and size of the litter. Since goats play a significant role in the tropical meat industry, growth efficiency and body weight (BW) variations are essential for achieving the required market body weight with good carcass composition (Yoseph Mekasha, 2007). Thus, taking into account linear measurements in selection indicators that have a strong correlation with litter size would be of the highest importance in a goat breeding program.

5.2. Genetic variation/polymorphism at GDF9 locus

Litter size is one of a major economically important reproductive trait in goat production. The low heritability estimates of reproductive traits indicated that high environmental effects will reduce the ability of phenotypic selection for improving such traits. Growth differentiation factor 9 (GDF9) belongs to the transforming growth factor β superfamily and plays a critical role in ovarian follicular development and ovulation rate (Elvin *et al.,* 1999). Polymorphism of GDF9 gene had been used as useful DNA markers for selecting high prolificacy individuals (Elieser *et al*., 2018). Understanding the genetic polymorphism of GDF9 gene and its association with litter size was pave the way for markers assisted selection in goat genetic improvement. In this study GDF9 gene polymorphism and its association analysis with litter size and body measurements were investigated for goats with different litter size (singleton, twin and triplet). Several studies reported the correlation between prolificacy and genetic polymorphisms in BMP15 and GDF9 genes in different goat breeds around the globe, including Black Bengal goat in India (Polley *et al.,* 2009; Hadizadeh *et al.,*2014; Ahlawat *et al*., 2015; Ahlawat *et al.,* 2016; Heikal and El Naby, 2017; Jalbani *et al.,* 2017; Mishra *et al*., 2017; Wang *et al*., 2017; Ghoreishi *et al.,* 2019; Wang *et al*., 2019; Yue *et al.,* 2019). This preliminary work is reported for the first time for Ethiopian local goats as it took the case of prolific goats of north shewa.

The analysis of variance showed that litter size was affected by body morphological traits of the does and genotype of GDF9 gene. Higher body morphological traits were observed in more prolific

does with triplet and twin. The same result was reported in Cameroon native goats (Kouam *et al.*, 2015). Body length, whither height and chest width and pelvic length showed high variation among the three litter size group.The result is in line with Haldar *et al.*, 2014) and Kouam *et al*, 2015) who reported that there was a strong and positive relationship between litter size and body length, withers height, chest girth in Indian and Cameroon goats, respectively. On the other hand, Mule *et al*, 2014) indicated that an increase in chest girth followed by increased body weight would simultaneously increase uterus volume to support an increase in the number and size of the litter. Hence, considering linear measurements having strong correlation with litter size in selection index would have a paramount importance in goat breeding program.

The observed allele 462 and 478, were with the range of the previous report in different goats breed which was 463 in Markhoz goat in Iran (Ghoreishi *et al*., 2019) 462 in Black Bangal and local Iraqi goats (Das *et al.*, 2021 Ali and Al-Azzawi, 2022),respectively. However, the new allele, 478, is higher than allele size, 471, that is reported in Indian goats (Ahlawat, 2013) which might be due to breed difference. The variation of allele frequency of GDF9 locus among does with different litter size in the present study is in line with the report of (Chu *et al*., 2011) who reported that there was a significant allele and genotype distribution among high and low prolificacy goats in China.
Estimates of mean H_E obtained in the present study were within the recommended range an average heterozygosity should be between 0.3 and 0.8 in the population to be used in measuring genetic variation (Nei, 1996). Effective and mean number of alleles at each locus gives information about the predominance of certain alleles in the populations. The average mean number of alleles reported for the current study is in line with the report of (Wang *et al*., 2010) for Funiu white goat. GDF9 locus was polymorphic in does having twin and triplet litter size. Alleles of polymorphic loci can be used as diagnostic marker to discriminate between prolific and non-prolific goat types (Chu *et al*., 2011). Besides, this result was also further confirmed by relatively similar average H_E value, close genetic distance and morphological similarity between does having twin and triplet compared to singleton.

5.3. Association of litter size with GDF9 gene polymorphism and body measurement

GDF9 gene polymorphism and its relationship with litter size were performed in goats such as Jining goats (Feng *et al.*, 2011),Black Bengal goat (Das *et al.*, 2021),Beetal and Teddy goats (Islam *et al.*,

2021),local Iraqi goats (Ali and Al-Azzawi, 2022). GDF9 was found to be associated with high prolificacy in local goats of the present study. All three genotypes showed positive association both in first and second parity. Genotype 478/478 showed strong association with only high litter size which was also evidenced by the result of body measurement association. The same genotype was associated with high value of body weight and liner body measurements and the same genotype is only found in goats have twins and triplets. The current finding was also supported by Abdelgadir *et al.,* 2021) and Ghoreishi *et al*., 2019) who reported that litter size was highly influenced by the genotypes of GDF9 gene and parities of goats.

The same result were reported for GDF9 heterozygous mutation was associated with high prolificacy in Guizhou White goat (Du *et al*., 2008) and homozygous mutation in Jining Grey goat (Chu *et al.,* 2011; Feng *et al.,* 2011). This might indicated that GDF9 gene mutation has a positive association with the specific genotype in prolific goats. The effects of body measurement on litter size result confirmed that those goats that have better body size had a chance to support number of litter size in does. However, we can not conclude that the identified polymorphisms correspond to other mutations than GDF9 gene which needs further investigation. Litter size of goat is very important trait for farm profitability and it is measured repeatedly in animal life with low heritability. Litter size is also affected by external factors such as nutrition, physiological condition and health which may not pass it down to the offspring (Zhu *et al.,* 2011). Application of traditional breeding methods, based on phenotypic performance, is time consuming process. Hence, selection of goats based on combining a higher litter size with fecundity gene mutation will enhance selection accuracy and breeding progress (Sun *et al*., 2010).

6. CONCLUSION AND RECOMMENDATION

6.1. Conclusion

The current study was designed to assess morphological variation and GDF9genepolymorphism indigenous goat population in North Shoa zone oromia regional state in darra district. Coat color pattern, hair type and coat color type significantly affected by location and agroecology hence, it is should be considred in phenotypic selection of goat and designing genetic improvement program. Most linear body measurements showed association with litter size of goat population. The body weight, length and chest girth of a goat with twins and triplets are higher compared to those with single births or small litters. In this study's morphological characterization indicated that most qualitative and quantitative traits in the indigenous goats varied significantly within and between individuals, and that variation was strongly correlated with litter size.

Significant ($p < 0.05$) and positive associations was observed in morphological traits and GDF9 genotypes among does with different litter size. Better genetic diversity indices and genetic polymorphism was observed in prolific goat that having twin and triplet litter size. Allele of GDF9gene which is 478 was unique from different study it can be useful to develop new breed program and morphological chaterization significantly associated whith higher litter size. The overall result suggests that GDF9 locus can be used as a promising diagnostic marker in the selection of prolific goats for productivity improvement in goat breeding program.

6.2. Recommedation

- The present study revealed morphological variation and high GDF9 gene polymorphism of exsit in indigenous goats with diffrent litter size. However, the current result is priliminare with a small number of goats in one district including more goats population with large number of individual goats and additional fecundity genes will be requrded to sustnitate the current result .
- Further analysis on PCR-RFLP-based identification of polymorphisms and sequence GDF9gene is required to identify point of mutation in Ethiopian goats population and study areas

- Further correlation of morphological variation and GDF9gene and other fecundity gene of indeginous goat can be used to improve best breed selection using fecundity gene marker than using only phenotyping /traditional breedingselection

7. REFERENCE

Abraham, Hagos, Solomon Gizaw. and Mengistu, (2017).Begait goat production systems and breedingpractices in Western Tigray, North Ethiopia. Open .*Journal of Animal Sciences**7**:198-212*

Ahlawat, S., Sharma, R., Roy, M., Mandakmale, S., Prakash, V.&Tantia, M.(2016).Genotyping of novel SNPs in BMPR1B, BMP15, and GDF9 genes for association with prolificacy in seven Indian goat breeds. *Anim. Biotech*.**27**:199-2017

Ajmone-Marsan, P., Colli, L., Han, J.L., Achilli, A., Lancioni, H., Joost, S., Crepaldi, P., Pilla, F., Stella, A., Taberlet, P., Boettcher, P.,Negrini, R., and Lenstra, J.A(2014).The characterization of goat genetic diversity:Towards a genomic approach. *Small Ruminant Research* **121**: 58-72

Ali, O., & Al-Azzawi, S. (2022).Detection of polymorphism in growth differentiation factor 9 gene (GDF9) Exon1 and its association with litter size in local Iraqi goats. *Revis Bionatur*,**7 (4)** 49.

Alubel Alemu, 2015).On-farm phenotypic characterizations and performance evaluation of Abergelleand Central highland goat breeds as an input for designing community-based breeding program. MSc thesis.Haramaya University, Haramaya, Ethiopia

Bambasi BoARD.Bambasi Bureau of Agriculture and Rural Development. Physical and socio-economic prole of Bambasi district. Ethiopia: Unpublished report. Bambasi; 2018

Biruh Tesfahun, 2013).Phenotypic and production system characterization of Goats in lowland areas of south Omo zone.MSc thesis, Submitted to the School of Graduate Studies of Haramaya University, Ethiopia.

Brahmi, A., Khaldi, R., Jaouad, M., Hicheri, A., Touati, I., Rkhissi, A., Ifaoui, H. and Khaldi, G., 2012). Impacts of climate change on the small ruminants farming systems in north western Tunisia and adaptation tools. New approaches for grassland research in a context of climate and socio-economic changes, **427**-431.

Bruford, Michael, Hanotte, Olivier, Brookfield, John, Burke,Terry, 1998).Single-Locus and Multilocus DNA Fingerprinting *J -Molecular Genetics Analysis of Populations:A Practical Approach*

Chu, M.X., Wu, Z.H., Feng T., Cao, G.L., Fang, L., Di, L., Huang, D.W., Li ,X.W. and Li, N, 2011).Polymorphism of GDF9 gene and its association with litter size in goats. *Vet. Res. Commun*. **35**:329-336

CSA (2018).Agricultural Sample Survey of 2017/18 (2010 E.C).Volume II Report on Livestock and Livestock Characteristics (Central Statistical Agency, Addis Ababa, Ethiopia

Das, A., Shaha, M., Gupta, M. D., Dutta, A., and Miazi, O. F, 2021.Polymorphism of fecundity genes (BMP15 and GDF9) and their association with litter size in Bangladeshi prolific Black Bengal goat. Trop Anim Health Prod, **53**:1-8.

DDLFDO (Derra District Livestock and Fishery Development Office).(2019).Annual Report of the Office.

Drouilhet, L., Mansanet, C., Sarry, J(2013).The Highly Prolific Phenotype of Lacaune Sheep

Dzama, K. (2016).Is the livestock sector in southern Africa prepared for climate change? South African Institute of International Affairs. SAIIA Policy Briefing 153

Ethiopia Biodiversity Institute (2016).Farm Animal Diversity of Ethiopia: Breeds and Ecotypes Catalo-gue. Ethiopian Biodiversity Institute (EBI), Addis Ababa, Ethiopia

Elieser S., Maharani, D., Budisatria, I,G., Batubara, A., Hariyono, D.N., Sari, A.P., (2018).Detection of Polymorphism In Growth Differentiation Factor 9 (GDF9) Gene In Indonesian Goats. In Proceeding of the 1st International Conference on Food and Agriculture.

Elvin J.A., Clark A.T., Wang, P., Wolfman N.M., Matzuk M.M., (1999).Paracrine actions of growth differentiation factor-9 in the mammalian ovary. J. Mol. Endocrinol., **13(6):**1035-48

FAO (Food and Agriculture Organization of the United Nations) (2020**).**World Food and Agriculture-Statistical Yearbook , FAO, Rome, Italy

FAOSTAT , 2016. Food and agricultural organization of the United Nations, statistical division

Farm-Africa (1996).Goat types of Ethiopia and Eritrea: physical description and management systems.London, UK, Nairobi, Kenya: Farm-Africa, International Livestock Research Institute (ILRI)

Feng,T G. L. Cao, M. X. Chu et al., “Identification and verification of differentially expressed genes in the caprine hypothalamic-pituitary-gonadal axis that are

associated with littersize,"Molecular Reproduction and Development,vol.82, no. **2,** pp. 132–138, 2015

Getent Mekuriaw, Mwacharo J.M, Dessie T, Mwai O, Djikeng A, Osama S, Girum Gebreyesus, Alayu Kidane, Solomon Abegaz, Kassahun Tesfaye (2017).Polymorphism analysis of kisspeptin (KISS1) gene and its association with litter size in Ethiopian indigenous goat populations. *Afr. J. Biotechnol*., **16(22):**1254-1264

Ghaffari, M.,Nejati –Javaremi,A and Rahimi,G (2009).Detection of polymorphism in BMPR-IB gene associated with twinning in Shal sheep using PCR-RFLP method .*International J. Agricultural and Biology*, vol.**11**:97:99

Ghoreishi,H., Fathi-Yosefabad,S., Shayegh,J., Barzegari,A. (2019).Identification of mutations in BMP15 and GDF9 genes associated with prolificacy of Markhoz goats. *Archives animal breeding*, **62**:565-2019

Solomon., Abegaz, S., Rischkowsky, B., Haile, A., Mwai, A.O. and Dessie, T(2013).Review of sheep research and development projects in Ethiopia. Nairobi, Kenya: *International Livestock Research Institute*

Gootwine, E. (2020).Invited review Opportunities for genetic improvement toward higher prolificacy in sheep. Small Rumin. Res., **186**, 106090

Groeneveld, L.F., Lenstra, J.A., Eding. H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Jianlin, H., Finlay, E.K., Groeneveld, E., Weigend, S. & the GlobalDiv Consortium. Genetic diversity in livestock breeds. (2010) .*Animal Genetics*, **41**: 6-31

Guan, D., Luo, N., Tan, X., Zhao, Z., Huang, Y., Na, R., Zhang, J.; Zhao, Y. (2016).Scanning of selection signature provides a glimpse into important economic traits in goats (Caprahircus). *Sci. Rep.***6**: 36372

Hadizadeh, M., Mohammad,R.M., Ali, N.,Ali, E., Yasser, M(2014).Search for polymorphism in growth and differentiation factor 9 (GDF9) gene in prolific beetal and tali goats (Capra hircus). *J. Bio. & Env. Sci.* **4 (4):** 186-191

Halima Hassen *et al.* 2012.Phenotypic characterization of Ethiopian indigenous goat populations. African Journal of Biotechnology. *Academic Journals* **11(73**): 13838-13846

Hanrahan, J.P., Gregan, S.M., Mulsant, P., Mullen, M., Davis, G.H., Powell, R and Galloway, S.M. (2004).Mutations in the genes for oocyte derived growth factors

GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (Ovisaries). *Biol. Reprod*. **70**: 900909

Helen Nigussie., Yoseph, Mekasha., Kefelegn, K., Solomon, A. and S.K. Pal (2013).Production objectives breeding practices and selection criteria of indigenous sheep in eastern Ethiopia. *Livestock Research for Rural Development***25** (9).

Hussein Hassen Toyib, 2015.Phenotypic characterization and breeding practices of ArsiBale goat population in selected districs of Arsi and Bale zones, Oromia regional state, Ethiopia.MSc thesis. Bahir Dar University, Bahir Dar, Ethiopia

Islam, M., Basheer, A., Javed, K., Anjum, A. A., and Zahoor, I. (2019).PCR-RFLP-based identification of polymorphisms in BMPR1B, GDF9 and BMP15 genes associated with litter size in Beetal and Teddy goats. S. Afr. J. Anim. Sci., **49(4):**697-708.

Jalbani, M.A., Kaleri, H.A., Baloch, A.H(2017).Study of BMP15 gene Polymorphism in Lehri goat breed of Balochistan. *J Appl Environ BiolSci***7(2)**:84-89

Khan, M.S., Muhammad, M.S. & Abdullah, M., 2013.Livestock Keepers' perception of indigenous goat breeds and their contribution to livelihoods in Pakistan. Egyp. J.Sheep Goat Sci. **8**, 17-28

Kouam, S. J., Meutchieye, F., Kenfack, W. P., and Manjeli, Y. (2015).Prolificacy and its relationship with body measurements in Cameroon native goats. Bull. Anim. Health Prod. Afr., **63(4):**235-241.

Mazhangara I.R.,, Chivandi E., Mupangwa F.J. and Muchenje V. (2019).The potential of goat meat in the red meat industry. Sustainability, **11(13):** 3671.

Melkamu Bezabih Yitbarek and Gebreyohannes Berhane (2014).Livestock Production Systems Analysis. Am. Int. J. Contemp. Res., **1(2):**16-51.

Mishra,C M. Rout, S. P. Mishra, S. S. Sahoo, G. Nayak, and R.C.Patra,"Genetic polymorphism of prolific genes in goat a brief review," Exploratory Animal and Medical Research, vol. 7, no. 2, pp. 132–141, 2017.

Monsivais, D., Matzuk, M.M. and Pangas, S.A. (2017) The TGF-β family in the reproductive tract. *Cold Spring Harb. Perspect. Biol* **9(10):** a022251

Muhammad, M.S., Abdullah, M., Khan, M.S., Javed, K. &Jabbar, M.A (2015).Farmers preferences for goat breeds in Punjab. Pakistan. *J. Anim. Plant Sci*. **25**: 380-386.

Nawaz, A., Babar, M.E., Hussain, T., Nadeem, A., Bilal, F., Javed, K. & Muhammad, K (2013).Identification of molecular markers in BMP15 gene of Pakistani goat breeds. *Int. J. Adv. Res* **1**: 62-68

Nei, M. (1996).Phylogenetic analysis in molecular evolutionary genetics. Annu. Rev. Genet., **30(1**):371-403.

Netsanet Zergaw.(2014) On-farm phenotypic characterization and performance evaluation of central highland and woyto-goji goat types for designing community based breeding strategies in Ethiopia. M.sc. thesis, Haramaya University, Ethiopia

Palmer K., Naicker A. and Kolanisi U. (2022).The Potential of Goat Meat Acceptance by Young Adults in South Africa. Afr. J. Inter-Multidiscip. Sud., **4(1):** 406-418.

Parajuli,A.K., Kolachhapati, M.R., Bhattarai,N (2015).Effect of non-genetic factors on reproductive performance of hill goat in Nawalparasi, Nepal. *Nepalese Journal of Animal Science* 29-40.

Pramod, R.K., Sharma, S.K., Singhi, A., Pan, S. &Mitra, A (2013).Differential ovarian morphometry and follicular expression of BMP15, GDF9 and BMPR1B influence the prolificacy in goat. Reprod. Domes. *Anim.***48**:803-809.

Ran, X.-Q.; Pan, H.; Huang, S.-H.; Liu, C.; Niu, X.; Li, S.; Wang, J.-F (2018).Copy number variations of MTHFSD gene across pig breeds and its association with litter size traits in Chinese indigenous Xiang pig. J. *Anim. Physiol. Anim. Nutr*.**102**,:1320–1327

Ray, S. K. Dash, S. K. Dhal, G. D. Nayak, and A. K. Parida, "Genetic studies on reproductive performance of indigenous goats in northern Odisha," Exploratory *Animal and Medical Reseach*, vol. **6**, pp. 192–198, 2016

Shapiro, B.I., Gebru, G., Desta, S., Negassa, A., Nigussie, K., Aboset, G., Mechal, H., (2015).Ethiopia livestock master plan. ILRI ProjectReport. Nairobi, Kenya: *International Livestock Research Institute (ILRI).*

SPSS I (2013).IBM SPSS statistics for windows. Armonk, New York, USA: IBM SPSS, 2.

Sun R ,Z., Lei, L., Cheng, L., Jin Z. F,, Zu ,S. J., Shan, Z. Y., Wang, Z .D., Zhang, J. X and Liu Z .H. (2010).Expression of GDF-9, BMP-15 and their receptors in mammalian ovary follicles. *J Mol Histol* **41**: 325–32

Tao,F.,Li, Z.Z.X.DNA(2011).polymorphism of introns1and 2 of Prolactin Receptor Gene and its association with litter size in goats. *Anim. Sci. Pap. Rep* **29**:343–350.

Tekeba Eshetie, Kelifa Hussien, Tadesse Teshome, Abebaw Mekonnen (2018).Meat production, consumption and marketing tradeoffs and potentials in Ethiopia and its effect on GDP growth: a review. *J. Nutri. Health Food Eng,* **8(3):** 228-233.

Tesfaye Tsegaye.2009.Characterization Of Goat Production Systems And On- Farm Evaluation Of The Growth Performance Of Grazing Goats Supplemented With

Different Protein Sources In Metema Woreda, Amhara Region, Ethiopia. M.Sc. Thesis, Haramaya University, Haramaya, Ethiopia, 129 pp

Thornton, P.K., 2010.Livestock production: Recent trends, future prospects. Philosophical Transactions of the Royal Society of London B: *Biological Sciences* **365:**2853–2867

Toro, M. A., T. H. E.,Meuwissen, J., Fernandez, I.,Shaat and A. Maäki-Tanila(2011).Assessing the genetic diversity in small farm animal population. *Animal,* **5**(**11**):1669-1683

Wang, X.Y., Yang, Q.,Wang, K.,Yan, H.L., Pan, C.Y., Chen, H., Liu, J.W., Zhu, H.J., Qu, L.,Lan, X.Y(2019).Two strongly linked single nucleotide polymorphisms (Q320P and V397I) in GDF9 gene are associated with litter size in cashmere goats. *Theriogenology***125**: 115–121

Yadeta Nemebergaga (2016).Production anreproduction performances, producers' trait preferences and marketing system of small ruminants in Ada barga and Ejere districts of west shoa zone, Ethiopia.

Yeh F, Yang R and Boyle T. (1999).Microsoft Window-based freeware for population genetic analysis (POPGENE), ver. 1.31. University of Alberta, Canada.

Yoseph Mekasha.(2007).Reproductive traits in Ethiopian male goats, with specialreference to breed and nutrition

Zewdu E., A. Haile, M. Tibbo, A. K. Sharma, J. Sölkner, and M. Wurzinger. (2012, Jul).Sheep production systems and breeding practices of smallholders in western and south-western Ethiopia: Implications for designing community-based breeding strategies".*Livestock Research for Rural Development*. Vol. **24**(7).

Zhang, C.Y., Chen, S.L., Li, X., Xu, D.Q., Zhang,Y. Yang, L.G 2009.Genetic and phenotypic parameter estimates for reproduction traits in the Boer dam. *Livest. Sci* **125**: 60-65.

Zhu, G.Q., Wang, Q.I., Kang, Y.G.,L.v,Y.Z., Cao, B.Y(2013). Polymorphisms in GDF9 Gene and Its Relationship with litter size in five breeds of Black goats. *Iranian Journal of Applied Animal Science*. **3 (3)**: 625-628

APPENDICE/ANNEXES

Appendix table 1. Description of qualitative traits observed for each sampled goat

Qualitative traits	Description
Coat color pattern	Plain, Patchy/pied, Spotted
Type of coat color	Red, brown,white, black, plain,spotted
Hair type	Smooth hair,Straight hair,glossy

Appendix table 2. Description of quantitative traits measured for each sample goat

Parameter	Units	Description
BW= Body Weight	Kg	The live body weight of goat taken using spring balance (in kilograms)
BL=Body Length	Cm	The horizontal distance from the pin bone to the point of shoulder to the nearest centimeter
WH=Wither Height	Cm	Height from the top of the withers to the ground
RH=Rump Height		Height from the top of the rump to the ground
CG=Chest Girth	Cm	Circumference of the chest in centimeters behind the shoulder of the body
CW=Chest width	Cm	The width of the chest between the briskets to the nearest Centimeter
CD=Chest Depth	Cm	Vertical distance from the top of the withers to the xyfoid process of the sternum measured to the nearest centimeter
PW=Pelvic Width	Cm	The horizontal distance between the extreme lateral points of the hook bone (tuber coxae) of the pelvis.
TL=Tail Length	Cm	The distance from the base of the tail to at the tip of the tail

Appendix table 3. ANOVA table of quantitative trait affected by different factors

Factors	SS	CV	R2	RMS	F value	P value	Signlevel
District							
BL	385	8.2	0.04	5.1	2.4	0.025	*
WH	455	7.6	0.056	4.79	3.3	0.003	**
CD	140	15.6	0.085	2.1	5.1	.0001	***
CG	458.9	9.9	0.029	6.8	1.65	0.13	Ns
TL	117.7	13	0.12	1.6	7.6	.0001	***
PW	357	12.1	0.30	1.59	23.5	.0001	***
PL	161.3	12.7	0.10	2.0	6.64	.0001	***
RH	219	7.19	0.02	4.7	1.64	0.13	Ns
Agro ecology							
BL	11.39	8.36	0.001	5.19	0.42	0.51	Ns
WH	45.3	7.7	0.005	4.88	1.9	0.16	Ns
CD	57.6	15.9	0.035	2.17	12.1	0.0006	***
CG	212.9	9.9	0.013	6.8	4.59	0.032	*
TL	14.9	13. 7	0.015	1.68	5.2	0.022	*
PW	88.2	13.8	0.07	1.8	26.69	.0001	***
PL	0.42	13.3	0.000	2.1	0.10	0.75	Ns
RH	13.6	7.2	0.001	4.7	0.61	0.43	Ns
Birth type							
BL	29.9	8.3	0.003	5.19	0.55	0.57	Ns
WH	109	7.75	0.013	4.87	2.29	0.10	Ns
CD	30.88	16	0.01	2.19	3.19	0.042	*
CG	24.3	9.98	0.001	6.8	0.26	0.77	Ns
TL	19.7	13.7	0.019	0.68	3.35	0.036	*
PW	330	12.2	0.27	1.6	63.7	.0001	***
PL	61.1	13.1	0.04	2	7.1	0.001	**
RH	36.1	7.23	0.004	4.7	0.87	0.44	Ns

Appendix table 4. ANOVA table of quantitative trait of only female

		Sum of Squares	df	Mean Square	F	Sig.
BL	Between Groups	569.387	6	94.898	3.031	.007
	Within Groups	8704.388	278	31.311		
	Total	9273.775	284			
WH	Between Groups	403.607	6	67.268	2.976	.008
	Within Groups	6283.537	278	22.603		
	Total	6687.144	284			
CD	Between Groups	147.398	6	24.566	6.903	.000
	Within Groups	989.388	278	3.559		
	Total	1136.786	284			
CG	Between Groups	897.237	6	149.539	4.663	.000
	Within Groups	8914.707	278	32.067		
	Total	9811.944	284			
TL	Between Groups	129.133	6	21.522	9.377	.000
	Within Groups	638.095	278	2.295		
	Total	767.228	284			
RL	Between Groups	369.714	6	61.619	26.697	.000
	Within Groups	641.655	278	2.308		
	Total	1011.368	284			
PW	Between Groups	153.533	6	25.589	6.301	.000
	Within Groups	1128.979	278	4.061		
	Total	1282.512	284			
RH	Between Groups	165.551	6	27.592	1.762	.107
	Within Groups	4352.645	278	15.657		
	Total	4518.196	284			