PHENOTYPIC, GENOMIC AND PHYSIOLOGICAL BASIS OF FECUNDITY TRAITS IN BONGA SHEEP OF ETHIOPIA

PhD DISSERTATION

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STATEMENT OF THE AUTHOR

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Dedication

To

My mother Wozgane Wolebo

My wife Etalem Akalewold Desta

My daughters Nuhamin and Ruth

My sons Tsegazeab (Murah) and Betemariam (Baby),

whose steadfastness, tenacity, faith, prayers and love will always fascinate me.

ABBREVIATIONS AND ACRONYMS

AFL	Age at first lambing
BMP15	Bone morphogenetic protein 15
BMPR1B	Bone morphogenetic protein receptor type IB
CL	*Corpora lutea*
EBV	Estimated breeding values
EHH	Extended haplotype homozygosity
ELISA	Enzyme-linked immunosorbent assay
FSH	Follicle stimulating hormone
GDF9	Growth differentiation factor 9
GO	Gene ontology
HP	High prolific
ICARDA	International Center for Agricultural Research in Dry Areas
ISGC	International Sheep Genomics Consortium
LD	Linkage disequilibrium
LH	Luteinizing hormone
LI	Lambing interval
LP	Low prolific
LS	Litter size
PCA	Principal Component Analysis
PPI	Functional protein-protein interaction networks
REML	Restricted maximum likelihood
TGFβ	Transforming growth factor β
XP-EHH	Cross population extended haplotype homozygosity

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LIST OF PUBLISHED PAPERS

This dissertation is based on the following papers referred to their roman numbers in the text.

I. Tera, A., Getachew, T., Melesse, A., Rekik, M., Rischkowsky, B., Mwacharo, J., Abate, Z. and Haile, A. 2021. Estimates of genetic parameters and trends for reproduction traits in Bonga sheep, Ethiopia. Tropical Animal Health and Production. 53:42. https://doi.org/10.1007/s11250-020-02445-w.

II. Tera Dolebo A, Melesse A, Porcu C,et al. 2020. Increased number of large non-atretic follicles and co-dominance effects account for high litter sizes in Bonga sheep. Anim Sci J. 91(1): e13384. https://doi.org/10.1111/asj.13384.

III. Asrat Tera Dolebo, Negar Khayatzadeh, Aberra Melesse, Mourad Rekik, Aynalem Haile, Barbara Rischkowsky, Max F. Rothschild, Joram M. Mwacharo. 2019. Genome-wide scans identify known and novel regions associated with prolificacy and reproduction traits in a sub-Saharan African indigenous sheep (*Ovis aries*). Mammalian Genome. 30:339–352. https://doi.org/10.1007/s00335-019-09820-5.

Phenotypic, Genomic and Physiological Basis of Fecundity Traits in Bonga Sheep of Ethiopia

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ABSTRACT

Three interrelated investigations were carried out with the objectives of estimating genetic parameters and trends of selection for Age at First Lambing (AFL), Lambing Interval (LI) and Litter size (LS); understanding the ovarian and endocrine changes and identifying the genetic basis of prolificacy in Bonga sheep, Ethiopia. Ten years data (2009-2018) on reproductive performance of Bonga sheep, managed by two communities involved in a community-based breeding program were used for genetic parameter and trend analysis. Data on the reproductive traits were analysed to evaluate the effects of breeding communities, season of mating, year of lambing, and parity by fitting fixed effect model of GLM procedures of SAS. Restricted maximum likelihood procedure of WOMBAT fitting univariate animal model was employed to estimate heritability, repeatability and breeding values. Thirty-one ewes were selected based on LS records and divided into two groups of high prolificacy (n=20) with LS ≥ 2 and low prolificacy (n=11) with LS =1. At a synchronized oestrus, follicular dynamics were determined using transrectal ultrasonography while plasma oestradiol concentrations were monitored throughout the induced follicular phase. Whole blood was collected from 95 animals (31 gave birth to single lambs, 33 to twins, 30 to triplets and one to a quadruplet) for genome analysis. Candidate regions under selection were identified using selection signature analysis performed on Ovine HD BeadChip data. Results showed that Bonga sheep had overall mean AFL, LI and LS of 453 days, 254 days and 1.43 lambs, respectively. Estimates of heritability for AFL, LI and LS were 0.015, 0.009 and 0.085, respectively. The repeatability estimates for LI and LS were low (0.109 and 0.196, respectively) indicating that environmental factors had contributed to the variation in these traits among parities. The genetic trends for AFL, LI and LS over the years were significant (p<0.01). Investigation of ovarian basis for prolificacy of Bonga sheep indicated that the mean number of large follicles was higher (p < 0.05) in HP (high prolific), 1.78 than in LP (low prolific), 1.0 ewes at day of oestrus (day 0). Prior to oestrus, more (p < 0.05) medium follicles were visible for HP compared to LP ewes. Plasma oestradiol concentrations were higher in HP compared to LP ewes (18.9 vs. 14.5 pg/ml; p < 0.05). Similarly, ovulation number was higher for HP than LP ewes (2.3 versus 1.28; p < 0.05) indicating that higher ovulation rates and litter size in Bonga sheep are evidenced by the previous presence of more large follicles and the existence of co-dominance effects. Analysis of selection signature revealed one strong selection signature on a candidate region on chromosome X spanning BMP15, suggesting this to be the primary candidate prolificacy gene in the breed. Besides, the analysis also identified several candidate regions spanning genes not reported before in prolific sheep but underlying fertility, immunity and reproduction in other species. The genes associated with female reproduction traits included SPOCK1 (age at first

oestrus), GPR173 (mediator of ovarian cyclicity), HB-EGF (signalling early pregnancy success) and, SMARCAL1 and HMGN3a (regulate gene expression during embryogenesis). The genes involved in male reproduction were FOXJ1 (sperm function and successful fertilization) and NME5 (spermatogenesis). It has been also observed that genes such as PKD2L2, MAGED1 and KDM3B within the candidate regions, which might have been associated with diverse fertility traits in males and females of other species. The results further confirmed the complexity of the genetic mechanisms underlying reproduction while suggesting that prolificacy in the Bonga sheep and possibly African indigenous sheep is partly under the control of BMP15 while other genes that enhance male and female fertility are essential for reproduction fitness. It is concluded that the well-structured community-based breeding programs of Bonga sheep have resulted in measurable genetic gains for reproductive traits. Besides, the existence of mutation is the causative effect for the phenotypic observed differences in growth of follicles and variability in litter size in Bonga sheep.

Keywords: *age at first lambing, Bonga sheep, lambing interval, litter size, heritability, ovulation rate, prolificacy genes, selection signatures*

1. GENERAL INTRODUCTION

Small ruminants contribute significantly to the subsistence, economic and social livelihoods of a large human population in smallholder production systems in developing countries (Kosgey and Okeyo, 2007). Increasing human population, urbanization and incomes, coupled with changing consumer preferences are creating more demand for these animals and their products (Delgado, 2005). With a large and diverse population of 33 million, sheep production in Ethiopia contributes substantially to the livelihood and income of the rural poor and the country at large (CSA, 2019). However, the sector faces several challenges in boosting productivity per animal.

The absence of appropriate breeding programmes has long been one of the reasons for low productivity per animal in developing countries (Scholtz et al., 2013). Hence, genetic improvement could be one of the means to bridging the productivity gap and contribute to reversing the challenges the sector faces (Haile et al., 2019a). The development of relevant breeding objectives and breeding strategies for livestock in general and sheep in particular for smallholder and pastoral production systems has been noted as an issue that has received little attention in the tropics (Kosgey et al., 2004).

Past efforts since the 1960's on genetic improvement of small ruminants in Ethiopia mainly focused on importation of exotic genetics and crossbreeding with local stock (Tibbo, 2006). However, these genetic improvement programs were unsustainable and produced no significant effects on sheep and goat productivity and the national economy at large. The major limitation is the lack of a clear and documented breeding and distribution strategy. This top down approach has very little consideration of farmers and pastoralists needs, perceptions, views, decisions,

indigenous practices, and active participation, from inception through to implementation (Haile et al., 2013).

Thus, there is a need for an alternative, cost effective breeding strategy suited to smallholder conditions that typically relate to low-input systems with farmers having a common interest to improve and share their indigenous stock from inception through to implementation (Mueller et al., 2015). Hence, community-based breeding programs (CBBPs) have now been suggested as viable option to bring about genetic gains that improve sheep productivity and ultimately enhance smallholder farmer's livelihoods (Haile et al., 2019a). This new approach has been tested in a few places with promising results, for instance with sheep and goat in Ethiopia; dairy goats in Mexico; llamas and alpacas in Bolivia and Peru; and sheep in Argentina (Haile et al., 2018). The programs increased the productivity and profitability of indigenous breeds without posing any threat to indigenous genetic resources diversity.

Community-based breeding programs were first introduced to Ethiopia in 2009 by the International Center for Agricultural Research in the Dry Areas (ICARDA) together with the International Livestock Research Institute (ILRI), the University of Natural Resources and Life Sciences, Vienna (BOKU), and the National Agricultural Research System in Ethiopia (NARS) (Haile et al., 2018). To date, the breeding programs in Ethiopia have directly benefited more than 18,000 people in 3,200 households in 40 villages with an average income increase of 20 per cent in the CBBP sites of Bonga, Horro, and Menz (Gutu et al., 2015; Haile et al., 2018). Farmers have also created 35 formal Breeders' Cooperatives, which have been able to build capital. The Bonga sheep CBBP is one of the most structured and successful with 16 functional cooperatives and more than 1700 members.

Genetic trend obtained as the mean EBV of cohorts of animals in a generation or born within a given time period is a standard measure, which is routinely computed to demonstrate the efficiency of selection programmes in livestock (Meyer et al., 2018). Furthermore, knowledge on genetic parameters including heritability and repeatability for economically important traits are crucial for the genetic evaluation, planning and implementation of selection programs (Safari et al., 2005).

Previous reports substantiated that environmental factors such as year of lambing, season of lambing, parity number, plane of nutrition, lamb survival, disease and parasite infection have significant effects on the reproductive and productive performance traits of ewes (José et al., 2016). Hence, quantifying and understanding the effects of non-genetic factors and devising mechanism to alleviate the negative effect of such factors should get due attention to raise production.

Accurate selection is one of the most important strategies to maximize production in animal breeding. However, the lack of estimates of genetic parameters that are necessary for the prediction of genetic gains has commonly been cited as an obstacle in the design and implementation of conservation-based selective breeding programs in the tropics. As a result, there are few reports on successful selective breeding programs in this region (Gizaw et al., 2007). Estimates of genetic parameters in a given production environment are necessary to determine the selection method used, to estimate the maximum genetic gain achieved and to obtain accurate estimates of breeding values. Most of the available studies in estimating the genetic parameters in sheep have focused on growth traits. However, there have been few estimates of genetic parameters for reproductive traits in sheep in Ethiopia.

Reproductive efficiency is the net biological achievement of all reproductive activities (Kutluca and Emsen, 2016). It is an integrated process encompassing both extra-ovarian signals like gonadotrophins and intra-follicular factors such as locally produced growth factors (Webb et al.,

2003) and environmental factors (Gurdeep et al., 2014). In sheep, reproductive efficiency is the product of fertility, prolificacy and the lambs' survival (Hristova and Stoycheva, 2019). Thus, reducing days of age at first lambing, increasing number of lambs per lambing and reducing the number of days for lambing interval and reducing lamb mortality are some of the most important indicators of increased reproductive efficiency and hence any genetic improvement program should critically consider these traits (Yadav et al., 2013).

Follicle development and ovulation rates are major determinants that influence reproductive performance (Manman et al., 2017) and largely influenced by both genetic and environmental factors (Kumar et al., 2013). Thus, understanding the pattern of follicle development is important for designing improved methods to manipulate reproduction in domestic animals (Evans, 2003). Indeed, successful ovulation requires developmentally competent oocytes released with appropriate timing from the ovarian follicle (Darryl et al., 2007). In this regard, sheep have proved to be a valuable model for the study of follicular growth and selection. Most sheep breeds have one or two ovulations but there is wide variation in ovulation rate among different breeds influenced by genetic background and the effects of age, season and nutrition (Montgomery et al., 2001).

Folliculogenesis in sheep occurs from puberty throughout adult life during which only a few follicles from a pool of several million will grow to an ovulatory size, and fewer still will ovulate (Carlos et al., 1997). Thus, several morphological, biochemical and physiological changes of ovaries occur during the estrous cycle (Sharma and Sood, 2019). The process of folliculogenesis will take around 6 months, with most of this time devoted to the growth of primary follicles to a diameter of 2.5 mm (Carlos et al., 1997). However, for a follicle to reach dominance, it requires the integration of a number of processes involving extra-ovarian signals and intra-follicular

paracrine as well as autocrine regulators whether a follicle will continue to develop or diverted into atretic pathways (Webb et al., 2003; Webb and Campbel, 2007). The preovulatory follicles usually derived from the large follicle population present at the time of luteal regression, but the sheep has the ability to promote smaller follicles if required (Carlos et al., 1997).

Ultrasonography uses high-frequency sound (ultrasound) waves to produce images of internal organs and other tissues. Transrectal ultrasonic imaging provides a means for repeated, monitoring and measuring of follicles larger than 2 mm, regardless of their depth within the ovary (Melesse, 2016; Sharma and Sood, 2019). Ovarian folliculogenesis in mammals from the constitution of primordial follicles up to ovulation is a reasonably well understood and time old mechanism. The use of sheep with genetic mutation affecting ovulation rate has provided exceptional tools in the field of female reproductive biology since 1980 when Piper and Bindon (1982) proposed that the exceptional fecundity of the Booroola Merino sheep might in part result from the action of a single major gene.

There are fecundity genes with major effect on ovulation rate and litter size in different sheep breeds. Many studies on the genetics of prolificacy highlighted the importance of three major genes affecting ovulation rate in sheep (Davis, 2005). These transforming growth factor beta (TGFβ) superfamily proteins are essential for mammalian fertility (Joy et al., 2012). These include BMPR1B or FecB on chromosome number 6 (Souza et al., 2001), GDF9 or FecG on chromosome number 5 and BMP15 or FecX on chromosome X (Hanrahan et al., 2004). Ewes that possess the naturally occurring mutations of both BMP15 and GDF9 generally demonstrated higher ovulation rates (Hanrahan et al., 2004) and are considered prolific. The knowledge of genes that are involved in ovulation rate and litter size and the effects they have provides useful information for breeding and selection on those traits. The Bonga sheep breed present good maternal characteristics and

markedly with good litter size. Moreover, the breed exhibits a very high variability of prolificacy within the population ranging from one to four (Gutu et al., 2015). This suggests the existence of an autosomal major gene for prolificacy segregating the same way as other known breeds. Thus, information about Bonga sheep breed in relation to reproduction and major genes affecting prolificacy is of practical interest.

1.1. General objective

To evaluate the genetic trends for reproductive traits of Bonga sheep kept under community-based breeding programs while characterizing ovarian and endocrine related changes and identify known and novel genomic regions associated with prolificacy and reproduction traits.

1.2. Specific objectives

- To investigate the effects of non-genetic factors on reproductive traits and estimate genetic parameters for some reproductive traits so as to assess genetic progress in reproductive traits in Bonga sheep.
- To characterize and compare follicular dynamics, ovulatory response and some ovarian endocrine attributes during the follicular and the leuteal phases in synchronized Bonga sheep with different retrospective average records for litter size.
- To identify known and novel genomic regions associated with prolificacy and reproduction traits in Bonga sheep.

2. LITERATURE REVIEW

2.1. Reproductive efficiency

Reproductive efficiency of the ewes, which is associated to the number of weaned lambs per dam and to the overall herd profitability, is considered as one of the most important factors in lamb production systems (Gbangboche et al., 2006). Environment influences reproduction in sheep strongly and understanding of environmental factors affecting reproduction traits are needed for successful animal improvement programs. Reproductive efficiency is measured by age at puberty, fertility, lambing rate, and length of breeding season. By most estimates, the heritability of reproductive rate is low, but breed differences exist. The aim of breeding programs in livestock species is to maximize the rate of genetic progress for economic traits. Efficiency of sheep production is conditioned by fertility (Petrovic et al., 2012). The rate of increase in all animal populations largely depends on the reproductive efficiencies of both sexes under the prevailing conditions. Reproductive efficiency is the net biological accomplishment of all reproductive activities like puberty, oestrus, ovulation, fertility, implantation, gestation and successful lambing, survival, growth after birth (Kutluca and Emsen, 2016) and others like nutritional, environmental and health factors (Rosa and Bryant, 2003; Melesse et al., 2013).

The productivity of the ewe flock is a direct reflection of reproductive efficiency. The total weaning weight of each ewe's lambs per each lambing is one of the best criteria for their reproductive performance. These reproductive efficiencies include female and male fecundities, the number of offspring per parturition (litter size), and the mothering ability of the female and the length of the reproductive life of both sexes. Thus, fertility, litter size and lamb survival are the components of the overall ewe reproduction traits affected by genetic as well as environmental

factors (Kumar et al., 2013). Hence, development of gene mapping techniques, and locating alleles that are responsible for the fertility of sheep began a new chapter in predicting and controlling the fertility of sheep (Petrovic et al., 2012).

2.2. Reproductive traits of economic importance in sheep

Reproduction rate and lamb growth are traits of economic interest that have a major influence in efficiency and profitability of sheep production (Montossi et al., 2013). On the other hand, factors of genetics, nutrition and management have also major role in influencing the fertility and the final successful sheep production (Petrovic et al., 2012). The economic importance of these traits mainly associated with ovulation rate and litter size (Notter, 2008). Generally, all traits of economic importance should be included in the breeding goal of livestock breeding programmes because genetic improvement of the traits contributing to lamb meat production is permanent, cumulative, cost-effective and sustainable (Hysen et al., 2015).

2.2.1. Age at first lambing

Age at first parturition is an important parameter in livestock productivity and shown to significantly influence lifetime reproductive performance in sheep (Schoeman et al., 1991). Early lambing is a good indicator of early sexual maturity in ewes. Age at first lambing is a common and easy-to-measure selection criterion related to the age at puberty. It is worth to mention that the younger the ewe at first lambing, the longer is its reproductive life, resulting in more offspring produced and faster return on investment (Short et al., 1994). In sheep it is affected by breed, husbandry and management practices. This trait has wide variation among African sheep. The AFL of African sheep seems to have wide variation and attributed to breed, husbandry and management

practices. Poor nutrition and disease can also lead to delayed AFL through limiting early animal growth. Age at first lambing is affected by season of lambing where lambs born in the rainy season attain their puberty age earlier than those at dry season due to comparatively better nourishment they received from their mothers who have access to abundant grazing in the rainy season **(**Salifu et al., 2018). Age at first lambing is one of the factors influencing lifetime reproduction and thus, recommended for inclusion in the selection program (Schoeman and Jaeneltte, 1991). Early sexual maturity is a desirable trait in sheep production systems. Ewes lambing for the first time at one year of age allow producers to cull infertile animals earlier and to distribute the maintenance costs of the ewe flock over a larger number of lambs (Kutluca and Emsen, 2016). Available reports for AFL for Ethiopian highland sheep indicated 471±6 days (Demeke et al., 1999). On the other hand, Mohammadi et al. (2011) reported that the average of first lambing age of Iranian Afshari breed estimated to be 691 days with significant effect of birth year and single or twin births on age at first lambing.

2.2.2. Litter size

Litter size is defined as the total number of lambs born per lambing in discrete numbers (1, 2, 3, 4 and 5), is the most economically important trait in lamb production (Olesen et al., 1995; Bromley et al., 2001) and main component of reproductive efficiency (Olesen et al., 1995). Furthermore, it also has an important indirect effect on the improvement of quantitative traits. Higher LS allows more selection pressure to be applied on other economically important traits (Shaat et al., 2004). Differences in LS contribute more to total lamb-weight weaned per ewe than growth rate of individual lambs.

Litter size can be improved by within breed selection and by combining prolific breeds in a cross breeding scheme. Because the heritability of LS is usually low, a selection on phenotype will be quite ineffective in improving litter size. Thus, the use of estimated breeding values using BLUP and including information from relatives will substantially accelerate genetic progress. The estimates of genetic parameters obtained from a threshold model for LS in different background indicates that the likelihood of improvement in reproductive efficiency through selection of these traits alone may result in slow genetic improvement in reproductive efficiency (Latifi et al., 2017). Traditionally LS is considered and evaluated as a trait of female. Litter size is a trait that depends on ovulation rate and is affected by the number of fertilized oocytes. Published reports for LS of different sheep breeds are also available indicating as being affected by different factors. Litter size for Finnish Landrace breed was estimated in a range of 2.32 to 2.76 lambs per ewe depending on lambing seasons (Sormunen and Suvela, 1999). The ewes born twin had more LS than singles affected by parity. For Iranian Afshari flocks, Mohammadi et al. (2011) reported the highest LS to be at sixth parity (1.25 lambs) and the lowest at first parity (1.09). Litter size in West African sheep (Musa et al., 2005), Djallonke sheep breed in West Africa (Gbangboche et al., 2006) and Dorper sheep under accelerated lambing system (Schoeman and Burger, 1992) were estimated to be 1.24, 1.4 and 1.4 lambs, respectively. In addition, LS of Menz sheep (Mukasa-Mugerwa and Lahlou-Kassi, 1995), Garole sheep and Garole×Malpura crosses (Kumar et al., 2006) were estimated to be 1.12, 1.95 and 1.60 lambs, respectively. On the other hand, Gutu et al. (2015) reported LS of Bonga sheep to be 1.62 at field condition from the recorded data. Prolificacy is a complex trait as described by Shorten et al. (2013) influenced by paternal and fetal effects. The low heritability and repeatability estimates imply that selection based on, improvement of non-genetic factors in the flocks such as the ewe nutrition before mating and during pregnancy can lead to improvement of

these characteristics. On the other hand, Varies et al. (1998) suggested that application of multi-trait model is most appropriate for estimation of genetic parameters for litter size.

2.2.3. Lambing interval

Lambing interval is defined as the interval between two consecutive parturitions. It has three phases: the gestation period, the postpartum anoestrus period, and the service interval. Lambing interval has an important influence on sheep production. It is one of the main components of reproductive performance which is affected by the breed and year of lambing (Niftalem, 1990), season and parity of ewes (Mengiste, 2008), type of management, nutrition, type of mating (Gbangboche et al., 2006). Reducing the generation interval is a key breeding strategy for increasing the efficiency of animal productivity.

The LI estimated for different breeds and sheep populations is available in literature. For Iranian Afshari flocks, Mohammadi et al. (2011) reported 306 days of LI while Musa et al. (2005) reported 207 days of LI for West African sheep. On separate report, Gbangboche et al. (2006) estimated 242 days LI for Djallonke sheep. The annual LI in Finnish Landrace ewes was reported to be 345 days (Sormunen and Suvela, 1999). In other study on Menz sheep reared in the highlands of Ethiopia, LI was reported to be 252 days (Mukasa-Mugerwa and Lahlou-Kassa, 1995). These variations in LI in the stated sheep breeds might be due to the effect of lambing year, lambing month, and the number of lambing, birth type, management system and nutrition. This might be because of the fluctuation of feed availability because of the variation in the amount and distribution of rainfall between years and seasons that has influence on herbage production and performance of ewe to come into heat early after lambing (Berhanu and Aynalem, 2009). Parity affects lambing interval that earlier parity ewes had the longest lambing interval than ewes with

later parities that might be associated to the longer time they take to recover their body condition after lambing for next reproduction (Mekuriaw et al., 2013).

2.3. Environmental factors affecting reproductive traits

The major part of the income in any sheep production system is supplied through lamb production (Ekiz et al., 2005). Environmental and management factors considered as non-genetic factors are very important for lamb production because they can modify the expected value of the phenotype (José et al., 2016). Favourable environmental conditions like good feeding and management have the same influence as selection and hence more multiple births occur under favourable conditions (Petrovic et al., 2012). Their effects can be predicted and sound management practices should be applied to avoid lower phenotypic expression of reproductive and productive traits.

José et al., (2016) described age of ewe, parity of ewe, plane of nutrition, condition of ewe and time of mating are physical environmental factors that affect productivity in ewes. Thus, it is important to increase the efficiency of sheep production by improving economic traits. Farmers give little attention to environmental and management factors that could improve the reproductive and productive performance of ewes. Also, its effects are not transmitted to the progeny and therefore may mask the genetic component as well as the expression of genetic material of the individual (Falconer and Mackay, 2006). Magaña-Monforte et al. (2013) have reported the effects of year, season, lambing and nutritional management on the reproductive and productive performance of sheep breeds in tropics. On the other report, Davoud et al. (2017) indicated the significant effects of environmental factors (year of lambing, type of birth, and maternal age at lambing) on all reproductive traits and pointed out that selection based on the number of lambs

born per ewe can be more effective than other traits in improving reproductive performance in Kordi ewes of Iran.

2.4. Genetic parameter estimates for reproductive traits

Reproduction traits, such as fertility, number of lambs born belong to threshold traits. These traits are not continuous in their phenotypic expression, but with a continuous genetic variability (Gianola, 1982). Knowledge of genetic variation and covariation among traits is required for both the design of effective sheep breeding programs and the accurate prediction of genetic progress from these programs (Safari and Fogarty, 2003). It is also crucial for the genetic evaluation and for choosing the best selection schemes (Safari et al., 2005). Hence, accurate estimates of genetic parameters are required to develop effective and comprehensive breeding objectives that encompass these diverse traits (Safari and Fogarty, 2003). The heritability estimates of these traits are rather low and reflect the generally small genetic variance. Low estimates for reproductive traits did not mean that there was no possibility for genetic improvement, but rather that the expected genetic gain was low if selection for these traits was also low (Ana et al., 2009). It reflects a proportionally greater influence of environmental effects, as well as a low genetic variability for fertility, litter size, lamb survival, lambing frequency, and other reproductive traits (Aguirre et al., 2017). Thus, the usage of the threshold analysis rather than the linear analysis would increase accuracy and eventually would speed up the slow response to selection (Mohammadi et al., 2012). Other reports indicate that there is highly significant genetic and phenotypic correlation between age at first fertile service and AFL which indicating that improvement in one trait will automatically improve the other trait (Khan et al., 2017).

Some researchers in separate studies have compared estimates of genetic parameters in reproductive traits for different sheep breeds indicated the importance of a threshold model with linear models estimating genetic parameters and genetic evaluation (Casellas et al., 2007; Mekkawy et al., 2010). Estimation of maternal effects and the corresponding genetic parameters considered inherently problematic. Selection for LS requires knowledge of genetic parameters of LS measured in different parities. Compared to linear models, non-linear models have disadvantages in goodness of fit or predictive ability and they are time-consuming in computation, which might be prohibitive for routine calculations (Hagger, 2000).

Many studies on sheep with different genetic backgrounds were conducted that were aimed at estimating the genetic parameters of LS in ranges (Mokhtari et al., 2010; Latifi et al., 2014). The differences in direct additive and maternal heritability estimates of the studied traits in various studies can be due to the type of model used, sheep breed, structure and volume of the data used, management used in livestock, and finally the application of various breeding strategies of sheep. Although phenotypic variation in reproductive traits is high, the heritability of these traits is low. Therefore, the response to selection for these traits will not be much. Thus, the availability of REML algorithms for analyses fitting an animal model including maternal effects, genetic or permanent environmental as additional random effects has made this task less difficult.

The CBBP for indigenous sheep in Ethiopia started after detailed and comprehensive studies since 2009. Comparison of sheep flock size owned by CBBP participants indicated larger flock sizes attributed to the improvements in reproduction of sheep as evidenced by the shortage of breeding rams solved by the CBBP (Gutu et al., 2015). The same study elucidated that the percentage of members of the CBBP in Bonga (72.5%) resulting in the litter size improvement because of efficiency in reproduction.

2.5. Genetic trends in reproductive traits

The evaluation of genetic trend gives an indication of breed direction as well as the rate of genetic improvement since the application of the program (Bosso et al., 2007). Thus, the success of a breeding program can be assessed by actual change in breeding value expressed as a proportion of expected theoretical change of the breeding value mean for the trait under selection. Studies on genetic effects for reproductive traits in sheep are scarce in tropical areas. Previous reports for genetic trends in growth and reproduction traits for Awassi sheep of Syria by Haile et al. (2019 b) emphasized the importance of systematic and organized selection scheme to obtain a change in the desired direction. Other report indicated low rates of genetic trends for reproductive traits explained by ineffective selection for reproductive traits due to the low heritabilities (Baneh et al., 2020). The genetic trends of reproductive traits in many studies elucidated that changes in performance must probably be by environmental condition improvement (Agurie et al., 2017). Thus, to substantially increase the genetic trends of traits in question in sheep breeding program, obvious breeding objectives, optimized breeding plan, suitable selection criteria, and accuracy of data collecting should be concerned because this information would be used in genetic evaluation program in order to achieve the maximum genetic trends under environmental conditions (China et al., 2013).

2.6. Endocrinology of reproduction

During 17-day estrous cycle, the uterus of the ewe is subject to morphological and functional changes with main events related to the periods of growth of the ovarian follicles and the *Corpus luteum* (AbuNasir and Aminoor, 2006). Uterine involution is a process that occurs in preparation

for the resumption of the estrous cycle and comprises three events: reduction in uterine size, tissue loss and tissue repair (Gray et al., 2003).

The interval between lambing and the onset of cyclic ovarian activity would allow a new conception to occur in a shorter period. As follicles develop following ovulatory stages, the sequence of events will occur in all species (Driancourt, 2000). Although various hormones are involved in the preparation of the ewe's uterus for conception, pregnancy mainly involves progesterone and estrogen (AbuNasir and Aminoor, 2006). Progesterone is the key hormone of pregnancy acting to prevent the resumption of cyclicity prepares the uterus for implantation and maintains myometrial quiescence (Lye, 1996).

Studies using transrectal ovarian ultrasonography in ewe lambs showed that antral follicle recruitment and growth increased after the first two months of age and just before puberty (Rawlings et al., 2003). In most sheep breeds, only one follicle ovulates at the end of each estrous cycle. Throughout reproductive life, different cohorts of follicles recruited to resume growth and development in follicle stimulating hormone (FSH) dependent process. The largest follicles in the cohort secrete high levels of inhibin A and oestradiol, causing decreased FSH concentrations. The early increase in antral follicle numbers and size in ewe lambs is due to changes in FSH release and potency, and enhanced follicle production prior to first ovulation caused by an increase in the frequency of the leutinizing hormone (LH) pulses (Rawlings et al., 2003).

Applications of exogenous hormones for increased reproductive performance in domestic ewes usually focus on estrous synchronization achieved by control of the luteal phase of the estrous cycle. The mechanism is either by providing exogenous progesterone or by inducing premature luteolysis. Methods based on progesterone or its analogues' effects in the luteal phase of the cycle, simulating the action of natural progesterone produced by the corpus luteum after ovulation

(Abecia et al., 2012). These devices would exert negative feedback on LH secretion that inhibit the endocrine events and lead to the maturation of pre-ovulatory follicles and ovulation (Jabbour and Evans, 1991). The ovarian cycle of the females endocrinologically represented by two phases: follicular and luteous. In the first phase, follicular growth takes place, where levels of blood progesterone descend to values lower than 1.0 ng/ml; in the second phase, progesterone increases due to ovulation and the formation of one or more corpora lutea, in this stage the progesterone reaches levels around 5.1 ng/ml (Montes-Perez, 2018).

2.6.1. Follicle development

For successful reproduction to occur, follicles must develop through several stages within the ovary that regulated largely by FSH and LH released from the anterior pituitary gland. During the advancement of follicle development, follicles increasingly respond to the actions of FSH and LH and hence decide its fate that depends on an intrafollicular balance between local factors that augment or attenuate gonadotrophic actions (Campbell, 2009). Thus, the diverse actions of FSH on follicles during the estrous cycle include stimulation of differentiation, hormone production, and proliferation of granulosa cells (Sullivan et al., 2013).

Earlier reports indicated that only a few follicles from a pool of several million will grow to an ovulatory size, and fewer still will ovulate (Carlos et al., 1997). Large pool of resting primordial follicles laid down during fetal development in sheep and goats, with the first follicles formed about 70 days of gestation (Mariana et al., 1991). Selection of the ovulatory follicle in sheep is a passive process where the largest follicle of the cohort of recruited follicles inhibits the FSH support to the other follicles via negative feedback action of its estradiol and inhibin (Driancourt, 1991).

In sheep, estradiol is involved in regulating the number of dominant follicles (Fortune et al., 1991). Dominance occurs in condition where largest follicles inhibit the growth of smaller ones. However, there are cases where follicles of 1-3 mm induce atresia of larger ones (Castonguay et al., 1990) as observed in Boroola sheep. The number of remaining (dominant) follicles is specific to the species and is indicative of litter size. In some prolific breeds, the high ovulation rate is achieved by the ovulation of follicles from the last two waves of the interovulatory interval (Bartlewski et al., 2011). This is achieved by more and smaller corpus luteum and lower serum concentrations of progesterone during the luteal phase of the oestrous cycle as compared to less prolific genotypes. Similar report by Gonzalez-Bulnes et al. (2004) suggested that increased ovulation rate in sheep carrying the FecB mutation related to a reduced rate of atresia with higher number of smaller diameter of follicles giving rise to smaller diameter of corpus luteum.

2.6.2. Wave-like patterns of follicular development in sheep

A follicle wave is the organized development of a cohort of gonadotrophin-dependent follicles all of which initially increase in size, but most of which subsequently regress and die by atresia (Evans, 2003). The development of ovarian follicles in sheep occurs in a wave like pattern with a predominance of three to four waves per inter-ovulatory interval for both prolific and non-prolific sheep breeds (Ali et al., 2006; Bartlewski et al., 2011). The number of waves per cycle affected certain patterns of follicular and luteal developments. This pattern of antral follicular development is closely associated with periodic elevations in daily serum concentrations of FSH; peaks of transient increases in daily FSH concentrations occur just prior to follicle wave emergence (Bartlewski et al., 2011).

The theory of dominance in sheep occurs as within each wave one follicle grew larger than the other; second, the emergence of successive waves only occur after the demise of the largest follicle in the previous wave, third; the small sized follicles showed peaks of growth at times constant with the follicular emergence (Ali et al., 2006). On the other hand, Bartlewski et al., (2011) described the fact that more than one follicle acquires the ability to reach an ovulatory size in a single wave and the follicles from two consecutive waves ovulate at the same time suggests that follicular dominance is weak or absent in the ewe. The follicles from two consecutive waves can ovulate together, especially in prolific ewes, and induced follicular waves do not suppress or delay the FSH peaks and subsequent waves of follicle growth (Bartlewski et al., 2011). Furthermore, studies of the hormonal control of ovarian cycles in ewes provide evidence for a rhythmic, endogenous firing of FSH peaks, whose periodicity and duration may be modified by luteal progesterone in cyclic ewes (Baby and Bartlewski, 2011; Bartlewski et al., 2011).

2.7. Ovulation in ewes

Ovulation rate is the number of matured oocytes released during one reproductive cycle. In mammals, it is determined by a complex exchange of hormone signals between the pituitary gland and the ovary and by a localized exchange of hormones within ovarian follicles between the oocyte and its adjacent somatic cells (Galloway et al., 2000; Eppig, 2001). Many mammals including goats and cattle normally have an ovulation rate of one or sometimes two (McNatty et al., 2005). The 17-day estrous cycle in ewes (day 0 considered as oestrus) is divided into a luteal phase lasting from days 2 to 13 and a follicular phase lasting from days 14 to 17. Progesterone based protocols are commonly used worldwide and administered by several methods, routes and doses to induce and synchronize oestrus and ovulation (Abecia et al., 2012). The basis to give intravaginal sponge

is to decrease LH secretion (Goodman and Karsch, 1980), which suppresses oestrus, LH surge and ovulation until the sponge removal.

Fertility is determined by the regularity of oestrus, the number and quality of ovulations and the incidence of embryo losses (Ben et al., 2010). Immunizations against either of these factors also results in similar increase in ovulation rate because of their major regulatory roles during both the gonadotrophin-independent and dependent stages of follicle development (Campbell, 2009).

Sheep carrying the FecB mutation have higher number and smaller diameter of pre-ovulatory follicles (Gonzalez-Bulnes et al., 2004). Another report also revealed that prolific breeds such as Finnish Landrace (Webb and Gauld, 1985) and Romanov (Driancourt et al., 1986) have reduced follicular diameter associated with a higher number of ovulatory follicles. Available evidences support that the fate of each follicle depends on the balance between stimulatory and inhibitory factors that modulate the role of gonadotrophins (Campbell, 2009). The most consistent characteristic of ewes carrying the prolificacy genes is the precocious development of a higher number of small ovulatory follicles associated with an earlier proliferation and differentiation of granulosa cells (Driancourt et al., 1985).

Ovulation rate and litter size are important reproduction traits in sheep and are of high economic value. Reproduction traits typically have low to medium heritability and do not exhibit a noticeable response to phenotypic selection. Therefore, inclusion of genetic information of the genes associated with reproductive ability could efficiently enhance the selection response (Yilong et al., 2018). Ovulation rate is also the primary source of variation in prolificacy, both within and between breeds (Webb et al., 2007). Thus, genetic improvement program aimed at increasing fecundity will ultimately improve the reproduction rate and production efficiency in sheep.

2.8. Ultrasonography

Introducing ultrasonography for diagnosing pregnancy and the number of fetuses in sheep gives new possibilities for increasing the efficiency of the reproduction (Bretzlaff et al., 1993). The use of ultrasonography has opened up the floodgates of research in the area of follicular dynamics as most efficient diagnostic tool for managing reproductive efficiency in small ruminants (Melesse, 2016; Sharma and Sood, 2019). With the advent of ultrasonography, our understanding of the dynamics and development of the follicle has increased in recent years. However, limitations associated with the use of ultrasonography include expertise of the operator and long time required to be trained on the technique (Gonzalez- Bulnes and Vazquez, 2010).

Ultrasonograpy technique in cattle has revealed wave-like cycles of selection, dominance, and regression of large antral follicles during the estrous cycle (Fortune, 1994). In sheep, however, the use of transrectal ultrasonography had faced difficulty to perform because of the anatomical access and smaller size difference between dominant and subordinate follicles and a random emergence of ovulatory-sized follicles, because of the fluctuation of FSH during the cycle (Carlos et al., 1997). The use of ultrasound scanning has increased the current knowledge of follicular and luteal function (Melesse, 2016) and, in fact, has triggered the revision of theories about patterns of follicular dynamics and existence and degree of follicular dominance. Evans (2003) described the high variability in the number of follicles developing in each wave and the high variability in the number of cohorts developing in each estrous cycle.

Bartlewski et al. (2002) examined the follicular growth pattern using transrectal ovarian ultrasonography every 2 weeks from 4 to 24 weeks of age in crossbred ewe lambs. The study revealed that numbers of antral follicles ≥3 mm of diameter increased from 14 to 16 weeks, decreased between 16 and 18 weeks, and then rose again between 22 and 24 weeks after birth with

maximum size of antral follicles peaked at 16 and 24 weeks of age. The same author reported the total number of all follicles ≥2 mm of diameter per ewe increased from 12 to 14 weeks, declined from 24 to 28 weeks, rose from 28 to 32 weeks, and then decreased after 32 weeks of age. The diameter of the largest follicle increased from 8 to 14 weeks, declined from 14 to 22 weeks, and finally increased between 32 and 38 weeks after birth (Rawlings et al., 2003). Increasing follicle numbers and diameters prior to the first ovulation is driven by an increase in the frequency of LH secretary pulses (Rawlings and Churchill, 1990).

The effect of follicular dominance exhibited in such a way that largest follicles in the cohort of each wave secrete high amounts of inhibin A and oestradiol, causing decreased FSH concentrations and the atresia of smaller follicles that inhibits the growth of existing and emergence of new follicles in the cohort (Fortune, 1994). Administration of exogenous FSH stimulates the growth of small follicles until pre-ovulatory stages and ovulation. On the other hand, rates of ovulation and embryo recovery are also related to the number of medium (4-5 mm) follicles at sponge withdrawal and to the number of large (≥ 6 mm) follicles at estrous behavior (Gonzalez-Bulnes et al., 2000).

2.9. Prolificacy

The term prolificacy is defined as the number of progenies born alive per parturition. It determines the efficiency of meat production in sheep (Bhuiyan and Curran, 1995). Fertility is often used as synonym of prolificacy although prolificacy is slightly different from fertility. Ewe fertility is combined trait controlled by genotype both the ewe and ram as the number of spermatozoa inseminated can affect prolificacy (Cameron et al., 1988). Nevertheless, to be prolific, an animal must be highly fertile (Musthafa and Marikar, 2014). There is linear relationship between fecundity

and prolificacy though the situation has been poorly understood in female animals (Vireque et al., 2008).

Generally, prolificacy is assessed as ovulation rate (number of mature oocytes released during one reproductive cycle), which is the primary source of variation in prolificacy, both within and between breeds (Webb et al., 2007). Previous reports indicated that variations in LS or ovulation rate in different sheep is associated with the segregation of several major genes (Mulsant et al., 2003). High fecundity reflects the high prolificacy. The selection aimed at increasing fecundity will ultimately improve the reproduction rate and production efficiency in small ruminants (Mishra, 2014).

The realized response to direct selection for prolificacy is number of lambs born per ewe joined per year and hence direct selection for LS in sheep has proved to be effective (Bradford, 1985). Thus, genetic improvement program aimed at increasing fecundity will ultimately improve the reproduction rate and production efficiency in sheep. Furthermore, an effective breeding plan can only be devised after thorough knowledge has been obtained about the inheritance of economically important traits like LS (Thiruvenkadan et al., 2008). The need to intensify sheep production to conform to today's systems of concentrated farming and to make sheep raising more economically rewarding to the keepers necessitated the use of prolific sheep to increase returns per unit of production.

2.9.1. Prolificacy genes

Davis (2004) described the current understanding of major genes affecting prolificacy in sheep falls into three categories. In the first category, mutation identified in genes and the DNA testing is available for them. These include BMPR-1B, GDF9 and BMP15. On the other category, mode

of inheritance of the genes described but mutation not been identified. These also include Woodlands gene, Thoka gene, and Lacaune. The other category is putative genes where there is evidence of apparent genetic segregation; but there are insufficient records to ascertain the mode of inheritance. This segment includes Olkuska, Belle-Ile and New Zealand Longwool breeds.

The Booroola trait was the first one noticed in Merino sheep producing large litters on the Booroola Farm in Cooma, Australia. Activin receptor like kinase (ALK6) was first found in Booroola ewes (FecB) at nucleotide position 830 (point mutation) leading to an arginine replacing glutamine amino acid (Q249R) in a highly conserved region of the intracellular kinase domain (Mulsant et al., 2001). It has been mapped in sheep chromosome 6 resulting in ovulation usually greater than 5 up to the extent of 15. A major prolificacy gene leads to twinning in sheep increasing ovulation rate by about 1.5 and 2 copies by about 3.0. These extra ovulations typically increased LS by about 1.0 and 1.5, respectively (Davis, 2004). Research and genetic knowledge indicated that location of the Booroola gene in native animals might offer an advantage over importation of breeding animals from other areas. Introgression of FecB gene via artificial insemination of non-prolific Malpura ewes using diluted semen of prolific sheep of Garole has resulted in improving the mean LS of crossbreds that are capable of adapting to a semi-arid tropical climate (Davendra et al., 2007; Kumar et al., 2006).

In Kalehkood sheep of Iran, BMPR-IB mutations were reported to be significantly associated with increased litter size (Morteza et al., 2014). Apart from the mutations at GDF9, BMP15 and ALK6 that have opened up many new paradigms for further research in this area, number of other genes in prolific sheep breeds yet to be recognized. Documented evidence also indicates that mutations in BMP15 increase ovulation rate in heterozygous carriers and block follicular development in homozygous carriers (Montogomery et al., 2001). A deletion in the BMP15 gene (GDF-9b) has

been recently shown to cause the Inverdale phenotype in ewes, which resulted in increased ovulation rate in the heterozygote and disrupted the follicle development of the homozygous carriers (Galloway et al., 2000).

Members of BMPs play a central role in determining ovulation quota and litter size (Kumar et al., 2013) suggesting that the segregation of a major gene affecting prolificacy (Demars et al., 2013). The identification of BMP15 and GDF9 gene mutations as the causal mechanism underlying the highly prolific or infertile nature of several sheep breeds in a dosage-sensitive manner also highlighted the crucial role these two genes play in ovarian function as well (Otsuka et al., 2011). These dramatically influence the number of ova ovulated in sheep (Kumar et al., 2013). Naturally occurring mutations in human and sheep of GDF9 (Wang et al., 2013) and BMP15 (Otsuka et al., 2011) and their receptors like ALK6 (Wilson et al., 2001) showed increases in ovulation rates in these species (Webb et al., 2016). Other reports also elucidate that member of the TGFβ superfamily (inhibins, activins and BMPs) work in concert with gonadotrophins throughout the follicular growth continuum (Webb et al., 2003)

Genetic screening for BMP15 in the sheep will help sheep farmers to optimize profitability through marker assisted introgression (MAI) of fecundity genes to directly manipulate litter size and also assists in the treatment of infertility/sterility in animals (Kumar et al., 2013). Incorporation of prolificacy genes into flock can be also achieved by marker-assisted selection, artificial insemination and embryo transfer programs (Davis, 2004). Access to DNA tested rams of different breeds carrying major genes for prolificacy allows breeders to choose the desired size of effect within a breed that is best adapted to their management conditions. The source of these mutations may be progeny tested or DNA tested rams carrying the major genes for prolificacy (Davis, 2005) with a desired size of effect within a breed that is best adapted to their management condition

(Davis, 2004). It can also be incorporated into crossbreeding program as well (Asha and Naicy, 2012).

Although numerous mutations improving reproduction traits have been discovered in various sheep breeds, the information whether or not genetic variants exist for sub-Saharan African prolific sheep breeds is almost nonexistent. There are very few studies on prolificacy (physiology and genetic) in African sheep breeds compared with sheep from Australia, New Zealand and Europe and as such this study presents novel information of interest to those working on African livestock. African livestock breeds are numerous, adaptive and diverse and have been used over centuries to provide livelihoods as well as food and nutritional security (Marshall et al., 2019). However, African breeds are unknown, their production traits are very little known, and researchers have not sufficiently addressed this area of the world where we have a large diversity of farm animal genetic resources on which the livelihood of millions of households depend.

2.9.2. Bonga breed as prolific sheep

Bonga sheep are native of Kaffa zone of Southern Ethiopia and one of the 14 indigenous sheep breeds of Ethiopia reared in highlands of Kaffa, Shaka and Bench Maji zones of Southern Ethiopia. The commonly used name 'Bonga sheep' was derived from the town of Bonga where the breed is marketed which is not familiar for the owners of the breed and earlier the sheep was known as the 'Kaffa sheep' that was bred during the administration of the last ruler of Kaffa, *King Tatto Gaki Sharoch* (Metsafe et al., 2017). They are of mutton type breed and have a higher body weight at maturity and the ewes are moderately prolific. The skeletal frame of the Bonga sheep is larger as compared to other Ethiopian sheep breeds and they are generally tolerant to many of the locally prevalent diseases (Haile et al., 2013). Bonga sheep breed was earlier considered as a subtype of

Horro sheep. However, the study conducted by Gizaw et al. (2008) indicated that the Bonga sheep is distinctly different from Horro sheep.

Bonga sheep breed is geographically distributed and reared by Kaffa, Sheka and Bench zones of Southern Ethiopia. The sheep is characterized by a long fat tail type with straight tapering end, large size and predominantly plain brown being both sexes polled (Gizaw et al., 2008). Currently, it has the total population of 725,572 (approximately 75% of total are females) of which 66% of them are being reared in Kaffa zone (CSA, 2019). However, it has been widely spread to the neighbouring areas of SNNPR and Oromia that share the same geographical boundary and market and are an important source of animal protein. The Ethiopian roasted sheep meat (*YeBeg Tibs*) is a major dish in hotels and restaurants and the '*tibs*' from Bonga sheep meat has exceptional taste and juicy nature. Personal observations indicate there is a quite considerable pile of meat in ribs of Bonga sheep not observed in other Ethiopian sheep. In areas like Kaffa where resources (particularly feed) are not constraints, availing breeding rams could result in more births and this could make significant contribution to improvement in livelihood of communities.

2.10. Community-based Bonga sheep breeding

Despite the large number and importance of adapted indigenous sheep breeds in the country, less emphasis has been given for their development. Breeding strategies implemented in developing countries in the past has been concentrated on the importation of higher-producing exotic temperate breeds developed for high-input, production environments, and often neglect desirable characteristics of indigenous breeds. In Ethiopia, crossbreeding of the indigenous sheep breeds with exotic breeds (Bleu du Maine, Merino, Rambouillet, Romney, Hampshire, Corriedale, and Awassi) made since early 1960 to improve growth and wool yield (Tibbo, 2006). However, such

genetic improvement programmes failed due to poor planning and due to the fact that they were implemented without considering all the needs of sheep owners and stakeholders in decision making and the program had no regard for the potential of indigenous breeds (Hassen et al., 2002; Kosgey et al., 2004).

Community-based breeding (CBB) is a farmer-participatory approach having common interest to conserve and improve their genetic resources under low-input production system (Mohammad et al., 2017). In 2009, ICARDA introduced community-based breeding program in Ethiopia. To date, the program has directly benefited 3,200 households in 40 villages with an average of 20 per cent income increase. Farmers have created 35 breeders' cooperatives to participate in the program. The government has identified CBBP as the strategy for genetic improvement of small ruminants in the Ethiopia Livestock Master Plan. The breeding cooperatives have been able to build capital from buying rams and bucks, as well as from other investments. For example, Bonga cooperative has a capital of around US$ 80,000. The program has been also implemented in other African countries like Uganda, Malawi, Tanzania, and South Africa. Hence, community-based breeding programs focusing on local genotypes advocated as the strategy of choice for genetic improvement of small ruminants (Haile et al., 2018). Currently, the government of Ethiopia recognizes the need for change in approach and community-based breeding programs identified as strategies of choice (Haile et al., 2013). Prior to CBBP several sheep genetic improvement projects initiated in Ethiopia focused mainly on importation of superior genotypes from abroad through top-down approach and hence were only able to partially meet their desired goals (Rege et al., 2010). Community-based breeding programs were designed and implemented for four sheep breeds of Ethiopia representing different agro-ecologies and production systems (Haile et al., 2018).

Community-based breeding program is carried out to conserve and utilize Bonga sheep breeds with active participation of farmers at Kaffa zone. Currently, the program implemented jointly by BARC and ICARDA at Boka and Shuta villages in 16 legally well-functioning cooperatives formed in the zone. Findings from these studies and the participatory research with farmers revealed shortages of breeding rams, inbreeding, and negative selections as some of the problems in sheep breeding practices. Based on the monitoring study conducted by Gutu et al. (2015), community-based breeding programs have achieved important outputs in reverting negative selection and acute shortage of breeding rams now rectified. Since the inception of the program, more births of lambs, large-sized lambs at birth and weaning and reduced mortality due to the combination of breeding with improved health care and feeding (Haile et al., 2020).

Steps for setting up CBBP

Figure 1. Logical sequence of steps to implement community-based sheep breeding (adapted from Haile et al., 2020)

Besides, negative selection has been reverted in a way that fast growing lambs retained for breeding rather than sold for market with an average genetic gain of 0.4 kg achieved per year. About 98% of the CBBPP participant farmers reported twinning (15% always twin, 72.5% mostly twin, 7.5% rarely twin and 2.5% rarely triple) since the inception of this program (Gutu et al., 2015).

Given the wealth of documented information on reproductive performance of sheep population in the tropics and the underlying genetic/physiological basis, it is of paramount importance to investigate the same for the Bonga sheep of Ethiopia. This would contribute to the already existing

knowledge base on prolificacy and lead to improvement of the Bonga sheep population. The present investigation was, therefore, undertaken with the following research questions:

- What are the non-genetic sources of variation affecting reproductive traits in Bonga sheep?
- What are the endocrine and ovarian changes that affect the litter size variability in Bonga sheep?
- What is the genetic basis of prolificacy in Bonga Sheep?

3. SUMMARY OF MATERIALS AND METHODS

This study involved both field and laboratory work. The fieldwork included cleaning and processing reproductive performance data collected over ten years from two Bonga CBBP communities (Boka and Shuta) in Southern Ethiopia. The study was conducted in two villages, Boka and Shuta of Adiyo Kaka district of Kaffa zone, Southern Nations' Nationalities and Peoples Region of Ethiopia. Boka-Shutta is located at 509 km South West of Addis Ababa. It is positioned at 7°17' N latitude and 36°24' E longitude and has a wet humid agroecology with an average elevation of 2511 m.a.s. level. The mean annual temperature ranges from 17.5 to 22.5°C. The area is covered by large evergreen natural forest receiving year-round rainfall.

1.1.Genetic parameters estimation and genetic trends

All phenotypic records collected over 10 year's period (2009 to 2018) were used to estimate genetic parameters and trends of the selection program undertaken at Bonga. Enumerators recruited by Bonga Agricultural Research center collected the data on the specified periods from Boka and Shuta breeder communities. The data used for this study consisted of 15,595 phenotypic observations from 1,500 ewes. The number of records varied for each trait (Paper I). Before conducting the main data analysis, screening for outliers was done. Pedigree viewer software version 6.5 (Birian and Sandy, 2015) was used to check for errors in the data such as duplicate animals, bisexuality, sire of itself and dam of itself. Factors considered as fixed environmental effects in the model included lambing season (based on rainfall distribution, two seasons of mating were established in the area and these include wet season (April to December) and dry season (November to March), ewe parity (1, 2, 3, 4, 5, 6, 7, and ≥8), year of lambing (2009-2018) and

CBBP sites (Boka and Shuta). Traits analyzed in the present study were AFL and LI in days and LS at birth in number. AFL and LI were calculated from the recorded information. The procedure of General Linear Model (GLM) of SAS 9.0 (SAS, 2009) was used to test the significance of fixed effects on reproductive traits. The Tukey–Kramer test was used to separate least squares means with more than two levels. Those effects that were found significant ($p<0.05$) in preliminary analysis were included in the model to estimate the genetic parameters. First order interactions were included in the preliminary analysis and were found to be non-significant and therefore were excluded from the final statistical analysis.

The model used for analysis of age at first lambing (AFL) was:

$y_{ijk} = \mu + S_i + A_j + E_k + eijk$, where

y_{ijk} = observations corresponding to AFL; μ = overall mean; S_i = effect of location (i = Boka, Shuta); A_j = effect of year of lambing (j = 2009-2018); E_k = effect of season of lambing (k = wet, dry); and eijk = residual effect, normal and independently distributed.

For litter size (LS) and lambing interval (LI), the statistical model used was:

$y_{ijkl} = \mu + S_i + A_j + E_k + N_l + eijkl$, where

y_{ijkl} = observations corresponding to LS; μ = overall mean; S_i = effect of location (i = Boka, Shuta); A_j = effect of year of lambing (j = 2009–2018); E_k = effect of lambing season (k = wet, dry); N_l = effect of parity of ewe (l = 1, 2, 3, 4, 5, 6, 7, and ≥8); eijkl = residual effect normal and independently distributed.

Estimates of (co)variance components and resulting genetic parameters (heritability and repeatability) were estimated by fitting univariate repeatability animal model using WOMBAT software (Meyer, 2007) applying the REML algorism. The animal model fitted was:

$Y = \mathbf{X}b + \mathbf{Z}a + \mathbf{W}pe + e$

Where: y = vector of observations for each trait,

b = vector of fixed effects,

a = vector of random animal genetic effects,

pe = vector of random permanent environmental effect of ewe,

e = vector of random residual effects, **X**, **Z** and **W** are incidence matrices relating records to fixed, animal genetic and permanent environmental effects, respectively.

3.2. Follicular and hormonal changes

To understand the physiological mechanisms underlying expression of litter size in Bonga sheep, a total of 31 non-pregnant ewes aged between 4 and 5 years and having a body condition score varying between 2.5 and 3.5 were selected from CBBP participant farmers of Boka–Shuta cooperative (Paper II). The ewes were chosen based on existing litter size records for three consecutive lambing seasons with no history of reproductive disorders. Ewes were classified as HP (high prolific, $n = 20$; average body condition score 3.1±0.56) producing litter sizes ≥ 2 and LP (low prolific, $n = 11$; average body condition score 3.0±0.42) producing litter sizes equal to one during each of the three considered lambing seasons. Throughout the experimental period, animals were collected and kept in a community shed, grazed on natural grassland and had *ad libitum* access to clean water. Three sexually mature rams of the Bonga breed were used for oestrus detection. Oestrous cycle was synchronized for the 31 selected ewes, using intravaginal progesterone sponges inserted for 14 days. Determination of follicular dynamics was performed daily by transrectal ultrasonographic assessment of the number and size of all follicles with ≥2mm, from the day of sponge removal to the day following the onset of oestrus.

Prior to echographic examination, all the ewes were subjected to 12-hour overnight fasting. The presence and number of CL were also assessed by transrectal ultrasonography approximately 9 days following the onset of oestrus. The same experienced operator using a 7.5 MHz transducer for transrectal ultrasonography (Honda®, HS-2200V; Tokyo, Japan) performed ultrasonographic observations of the ovaries. After placing the sheep in the dorsal position as during laparoscopy, the probe was placed in the rectum with the transducer orientated perpendicularly to the abdomen wall using a hydrosoluble contact gel to enhance ultrasound transmission. The probe was rotated laterally 90° clockwise and 180° counterclockwise to observe both ovaries and their structures after surpassing the urinary bladder. Each ovary was scanned several times from different angles in order to image all follicles with a size of ≥2 mm. The largest diameter of each of these follicles was measured and its position was recorded on a paperback diagram of each ovary. Follicles recorded by ultrasonography from the left and right ovaries were classified as small [2-3 mm], medium [4-5 mm] and large [≥ 6 mm] and total follicles.

Around day nine following the onset of oestrus, the left and right CLs were identified through their echogenic pattern and their numbers determined. Sixteen hours following the removal of the intravaginal sponges, oestrous behaviour was detected at 8-hour intervals via direct observation of the ewes using three aproned teaser rams. Oestrous detection continued for four consecutive days or until oestrus was detected. Ewes standing to be mounted were considered to be in oestrus and were mated with rams allocated at a mating ratio of 10:1. Each ewe was mated twice at 12 hours interval. Ewes not displaying oestrus were also recorded.

Blood sampling for oestradiol determeination took place every 8 hours, from 16 to 96 hours after sponge removal. This corresponded to the time-period during which follicular dynamics and ostrus behavior were monitored.

Blood was collected using vacutainer tubes coated with heparin. For progesterone analysis, blood smaples were collected at 48-hr intervals for 20 days following the removal of sponges. Samples were immediately placed in a cooling box filled with ice and then transported to Bonga Research Center and centrifuged at 1500 *g* for 15-20 min. Plasma, recovered from each centrifuged sample, was stored at -20°C for 3 weeks prior to undertaking progesterone and oestradiol assays. Plasma progesterone and estradiol concentrations were determined by ELISA in duplicate using an ELISA assay kit (MyBioSource®, San Diego, USA) based on standard procedures following the manufacturer's instructions. The respective inter- and intra-assay variation coefficients were 8.6% and 11.2% for progesterone and 6.2% and 10.3% for estradiol. For uniformity of variables, day 0 was equaled to be the day of onset of oestrous. Day 0 in ewes that did not show oestrus was assimilated to day 2 after the introduction of teaser rams on which more than 90% of the ewes displayed oestrus. For changes in the frequency of follicular size, factorial ANOVA with two independent factors (time and prolificity) was used to test the difference between LP and HP ewes. The Students *t*-test was used to compare differences in follicular numbers between the LP and HP ewes. One-way ANOVA was used to test differences in the number of CLs, atretic follicles, new follicles, and plasma concentration of oestradiol and progesterone between the LP and HP ewes. Mean number of small, medium, large, total, atretic and, new follicles were expressed as mean ± SEM. Mean plasma oestradiol and progesterone concentrations were expressed as mean ± SD. Statistical significance was set at $p < 0.05$.

3.3. Genotyping

To identify candidate genomic regions and genes associated with prolificacy, 95 ewes (31 gave birth to single lambs, 33 to twins, 30 to triplets and 1 to a quadruplet) belonging to the Bonga sheep were sampled from Boka and Shuta Bonga sheep breeder farmers in Southwestern Ethiopia (Paper III). All the ewes had at least three lambing parities and came from farmers flocks that are participating in the CBBP where performance recording was undertaken. Whole blood sample was collected from each animal via jugular vein puncture with EDTA coated test tubes as the anticoagulant. This was later transferred to Whatman™ FTA™ Classic Cards for storage. Genotyping was done using FTA™ preserved blood samples with the Ovine Infinium® HD SNP BeadChip that include 606,006 genomic variants designed by the ISGC, nearly all the contents from the original OvineSNP50 array and 30,000 putative functional variants.

Prior to performing selection signature mapping, the genotyped individuals were classified into two groups, prolific and non-prolific ewes. The prolific group included ewes with twins, triplets, and quadruplet litter sizes while the non-prolific group included ewes with single litter sizes. To identify candidate genomic regions under selection, three selection detection tests: F_{ST}, hapFLK and XP-EHH, were implemented.

To identify loci under selection, the allele frequencies retained SNPs for the two contrasting groups of prolific and non-prolific ewes were calculated. The allele frequencies were used to calculate F_{ST} values for each locus as a measure of group differentiation. For each SNP, F_{ST} was calculated as the squared deviation of the average frequency in a group from the average frequency across the groups divided by the allele frequency variance (p*q). To identify regions under selection, the non-prolific group was compared against the prolific one and the pairwise group values were averaged

to obtain a single F_{ST} value per SNP for each group. Smoothed F_{ST} values greater than the average plus/minus three standard deviations (mean $F_{ST} \pm 3$ SD) were taken to be under selection.

As a complementary approach to mapping selection sweeps, hapFLK 1.3 (https://forge-dga.jouy.inra.fr/projects/hapflk) was used. To perform hapFLK analysis, Reynolds' genetic distances between the prolific and non-prolific ewes were calculated and converted to a kinship matrix with an R script provided by hapFLK developers (available at https://forge-dga.jouy.inra.fr/projects/hapflk/documents). Subsequently, by assuming 10 haplotype clusters in the LD model (-K 10; number of haplotype clusters determined by running a fast PHASE cross-validation analysis), the hapFLK statistics were computed and averaged across 20 expectation-maximization runs to fit the LD model (–nfit = 20). The standardization of the statistics using the corresponding python script provided with the software allowed the estimation of the associated *P* values from a standard normal distribution.

The SelScan package (Szpiech and Hernandez, 2014) was used to perform an additional analysis based on the cross-population XP-EHH test (Sabeti et al., 2007). It is calculated as:

$$\text{Unstandardized XP-EHH} = \ln(iHH_A/iHH_B), \text{ where}$$

iHH_A and iHH_B are the integrated EHH of a given core SNP in population A and B, respectively. The software developed by Pickrell et al., (2009) was used to estimate the unstandardized XP-EHH statistics using all the SNPs that were retained following quality control. Positive and negative XP-EHH estimates indicated positive recent selection in prolific and non-prolific ewes, respectively. For consistency with the threshold used for hapFLK, those positions showing *P* values less than 0.001 were considered as significant.

For functional annotation of the candidate regions under selection for the three selection mapping approaches, positions that showed evidence of selection (mean $F_{ST} \pm 3$ SD; or showing a *P* value

less than 0.001 for hapFLK and XP-EHH) were considered to be the result of selection sweeps. The genes that were either partially or fully covered by these regions were identified based on the ovine 3.1 reference genome assembly using Ensembl Comparative Genomics Resources Database Release 94 (https://www.ensembl.org/index.html). Functional annotation and enrichment analysis for the candidate genes was performed with the functional annotation-clustering tool of DAVID Bioinformatics Resources 6.8 (Huang et al. 2009a, b). Each gene was analysed and enrichment analysis was performed using *Ovis aries* as the target species and the *Bos taurus* genome supplied with DAVID 6.8 as the background species. For the functional annotation clustering, an enrichment score of 1.3 was taken as the threshold following the authors of DAVID 6.8. A search of the literature was also performed to identify phenotypes that are known to be affected by variation in the genes found in the candidate regions in other species. Protein-protein interactions (PPI) and gene ontology (GO) enrichments were investigated with STRING (Szklarczyk et al., 2015). Functional protein-protein interaction networks and gene ontology terms encoded by the candidate genes were also investigated using STRING Genomics 11.0 (Szklarczyk et al., 2019) with the *Bos taurus* as the background species. A global PPI network which retained interactions with a high level of confidence (PPI enrichment score > 0.4) was constructed.

4. SUMMARY OF RESULTS

4.1.Effects of non-genetic and genetic factors on reproductive performance traits

4.1.1. Non-genetic factors

Overall average for age at first lambing (AFL), lambing interval (LI) and litter size (LS) for Bonga ewes in the present study were 453 days, 254 days and 1.43, respectively (Table 2; Paper I). The effects of non-genetic factors including breeding communities (sites), lambing year, lambing season, and parity of the ewe on all reproductive traits were significant ($p< 0.001$) except for that of site on AFL (Table 2; Paper I). Boka communities had higher LS and shorter LI than those of Shuta ($p<0.001$). Ewes of Boka lambed nearly 8 days earlier (251) than those of Shuta (259). Furthermore, ewes of Boka produced more lamb crops (1.47) than those of Shuta (1.39). Although Boka ewes gave birth to lambs at slightly younger age (453) than Shuta (461) ewes, the difference was not significant (Table 2; Paper I).

The year of lambing had significant effect on traits analyzed with wide fluctuations from year to year although it did not follow any regular pattern. There was a decreasing trend in LI until 2015 from the onset of selection with very slight pick in 2013. Similarly, LS had no regular pattern across the lambing years although lower values were observed in ewes that lambed in 2014 and 2017. Ewes that were mated during the wet season (April to December) had significantly shorter LI (245) than those mated in dry season (November to March). Similarly, ewes mated in wet season had higher number of lambs (1.46) than the other ones mated in dry season (1.39). Ewes that were mated in wet season also had shorter AFL (443) than those mated in dry season (471). Ewe parity showed significant effect on the traits investigated but did not follow regular pattern. There had been improving trend in LI as parity advanced from parity 2 to parity 4 and above. On the other

hand, the order of ewe parity had pronounced effect on the number of lambs born per ewe with the highest litter size at parity six (Table 2, Paper I).

4.1.2. Genetic parameters

Estimates of (co)variance components, heritability, and repeatability of the studied traits are described in Table 3 (Paper I). The direct animal variance components for AFL, LI and LS were 176.1, 21.2 and 0.020738, respectively. As a result, the heritability estimates for AFL, LI and LS for Bonga sheep were 0.015, 0.009, and 0.085, respectively. The repeatability estimates for LI and LS were 0.109 and 0.196, respectively. The number of lambs born had relatively higher heritability and repeatability than other traits under investigation.

4.1.3. Genetic trends

The genetic trend for LI, AFL and LS over the years was significant ($P<0.01$; Figures 1, 2 and 3; Paper I). While the trend was positive for LS, understandably, there was a decreasing trend for AFL and LI. The estimated breeding values (EBV) for LS has increased over the years although the pattern is not regular. Until 2011, the trend was very slow showing sharp increase from 2011 to 2012 and then a decreasing trend after year 2017. Similarly, the genetic trend for LI has shown decreasing trends over the years but did not follow regular pattern. The irregular patterns were mainly reflected in the years 2009 to 2010 and 2012 to 2013. The EBV for AFL also showed a decreasing trend over the years in general but showed increasing trend from years 2014 to 2015 and dropping sharply after 2016.

4.2.Follicular dynamics study

4.2.1. Oestrus response and growth dynamics of preovulatory follicles

Following sponges' removal and introduction of rams, 27 out of 31 ewes were detected in oestrus. Nine out of the 11 ewes (82 %) belonging to the group LP were detected in oestrus and 18 out of the 20 HP ewes (90%) were observed standing to oestrus. Changes in frequency of follicular size (mean number of small, medium, and large follicles) were represented in figure 1 (Paper II). Although it is not significant ($p = 0.07$), the average number of medium follicles tended to be higher in the HP group than the LP group. In fact, in the HP group, the mean number of medium follicles increased from 0.89 three days before oestrus to 4.89 and 3.89 at days -2 and -1 before oestrus, respectively (Figure 2, Paper II). Corresponding values in LP animals remained much lower (1.17, 2.83 and 1.83 at days -3, -2 and -1 before oestrus, respectively) (Figure 2, Paper II). Differences between LP and HP groups were significant on days -2 and -1 prior to oestrus ($p < 0.05$). However, for small and large follicles, no significant differences were observed between the two groups of ewes except on the day of oestrus (day 0) when the mean number of large follicles was higher in HP compared to LP ewes (1.78 and 1 for HP and LP group, respectively; $p < 0.05$) (Figure 1, Paper II).

4.2.2. Atretic and new follicles

No differences were observed in the trend follicles are undergoing atresia. Average number of new follicles was significantly higher in the ovaries of LP compared to their HP counterparts on the day of oestrus and on the day prior to oestrus ($p < 0.05$; Figure 2, Paper II).

4.2.3. Plasma oestradiol

Throughout most of the sampling period, HP ewes had higher means of plasma oestradiol but at some sampling points differences failed to reach statistical significance because of high individual variations (Figure 4, Paper II). Overall, plasma oestradiol concentrations were significantly higher ($p< 0.05$) for HP group in comparison to LP ewes (18.91 vs. 14.51 pg/ml).

4.2.4. Luteal function and plasma progesterone

Mean number of CL was significantly higher ($p<0.05$) in HP ewes (2.3) than those of LP (1.28). Mean plasma progesterone concentrations were higher ($p < 0.05$) in HP than LP ewes (Figure 4, Paper II) and these differences appeared to be more pronounced between days 10 and 15 after removal of sponges. The largest difference was observed on day 12 when progesterone concentrations rose to an average level of 5.60 ng/ml in HP ewes in comparison to only 1.95 ng/ml for LP ewes.

4.3. Genome wide scans of selection signatures

Principal component analysis (PCA) and net view using the retained variants detected no genetic stratification (Figures 1a and 1b, Paper III) with the first principal component of the PCA explaining 80.9% of the total genetic variation. Irrespective of their prolificacy (twinning, triplet, quadruplet), the entire ewes clustered close together with only eight outliers (five ewes with triplets and two with singlets) being observed.

Based on the ovine RefSeq gene annotation, five and eight candidate regions revealed by XP-EHH and hapFLK, respectively that overlapped no gene(s) (Supplementary Table 1). For the candidate regions that overlapped with gene(s), two (one on Oar5 and the other on OarX; Table 1; Figure 2a;

Paper III), were identified from the empirical genome-wide distribution of F_{ST} values. The region on Oar5 spanned 18 annotated genes and 5 novel protein-coding transcripts, while the one on OarX, the most significant signature, spanned eight annotated genes and seven novel protein-coding transcripts (Table 1; Paper III). The XP-EHH detected 18 candidate regions, spanning 20 annotated genes and 4 protein-coding transcripts, across 12 chromosomes (Table 1; Figure 2b; Paper III). The hapFLK, revealed 21 candidate regions spanning 31 annotated genes and 13 protein-coding transcripts, across 15 chromosomes (Table 1; Figure 2c). The candidate region on OarX overlapped between F_{ST} and hapFLK tests. The GDF9 gene occurred at 4,456,484 bp downstream of the candidate region revealed by F_{ST} on Oar5. None of the three candidate regions identified by XP-EHH on Oar5 overlapped with *GDF9* or the F_{ST} region.

In total, 73 annotated genes were observed in 28 candidate regions that were identified to be under selection by F_{ST} (2 regions), XP-EHH (18 regions) and hapFLK (21 regions). The top two clusters were associated with immune responses encompassing i) Toll/Interleukin-1 receptor homology (TIR) domain (enrichment score = 2.82) and ii) Immunoglobulin/Immunoglobulin-like (IG) domain (enrichment score = 1.27). The network proteins encoded by the 73 candidate genes had significantly higher interactions amongst themselves than was expected for a random set of proteins of similar size drawn from the genome (33 edges identified; PPI enrichment P-value = 0.00612; Figure 3). STRING revealed three GO biological process terms that were the most enriched (Supplementary Table S2). The PFAM, InterPRO and SMART Protein Domains were all associated with Toll/Interleukin-1 receptor (TIR) domain superfamily (Supplementary Table S3) while the SMART Protein domains included immunoglobulin-like domains as one of the most enriched. The Reactome Pathways were associated with Interleukin-18 signaling (BTA-9012546; False discovery rate = 0.0492). Apart from *BMP15* and *GDF9*, that are known to be associated

with prolificacy across a wide range of prolific sheep in Europe and the Middle East, functional annotation analysis identified several candidate genes associated with female and male fertility and reproduction functions (Table 1) in other species but which have not yet been reported in prolific sheep.

5. GENERAL DISCUSSION

5.1. Fixed effects

5.1.1. Age at first lambing

Reproductive efficiency of sheep flocks depends largely on the genotype in use (Gootwine, 2016). The AFL is an important reproductive trait as greater population turnover and more rapid genetic progress can be obtained when sheep produce their first offspring at an earlier rather than later age. As reported by Ayele and Mengistu (2019), early maturing females are known to have a relatively long and fruitful reproductive life. The AFL of African sheep has wide variation due to breed, environmental and other factors. It depends in part on the onset of puberty and the reproductive and nutritional management (Ramón-Ugalde et al., 2002). The AFL for Bonga ewes in the present study was estimated at 453 days, which is affected by lambing year and mating season but not by breeding communities. Ewes that were mated in wet season and hence lambed in September to May have attained their AFL 28 days earlier than those mated in dry season.

The average AFL of Bonga ewes managed by farmers in the present study is somehow higher than expected and this might probably be due to the small size of the data used. The present estimate is comparable with those reported 457 days for Ethiopian Washera sheep (Mekuriaw et al., 2013). On the other hand, Demeke et al. (1999) and Getachew (2008) reported about 470 days of AFL for the Ethiopian highland sheep and Menz sheep, which is higher than that found in the present study. Lakew et al. (2014) reported 469 and 555 days of AFL for Dorper sheep crosses and local Tumelie sheep of Ethiopia, respectively, which is far higher than the present study. Moreover, much higher values were reported by different authors including Yadav et al. (2018) and José et al. (2016) who respectively reported 713 and 526 days for Munjal Sheep and Pelibuey ewes in

Southeastern México. The lower AFL for Bonga sheep in the present study might be explained by the genetic differences and management of the well-structured community-based breeding program.

5.1.2. Lambing interval

The LI is also one of the determinant factors in sheep reproductive performance. Ewes reared in Boka communities lambed 8 days earlier than those of Shuta. This might be attributed to the level of awareness of farmers in animal management and consistency and accuracy in data collection and handling by the enumerators. Mavrogenis (1996) and Mohammadi et al. (2012) have reported significant effects of lambing year on reproductive performances. The significant influence of lambing year in the present study was attributed to variation in the climatic conditions leading to differences in pasture availability, management and breeding conditions of ewes and lamb feeding in different years that cause fluctuations in the reproductive performance. Previous reports on effects of non-genetic factors on LI in Ethiopia also confirmed the effects of breed and year of lambing (Niftalem, 1999); season and parity of ewes (Mengiste, 2008) and type of management, nutrition, and type of mating are factors that influence LI (Melesse et al., 2013).

In the present study, first parity ewes had the longest LI. As the ewe's parity advanced, LI was shortened; however, the differences were not significant after parity 4 and above. This observation is in close agreement with previous reports for Pelibuey ewes managed in humid environment in Mexico (Luna-Palomera et al., 2019), which could be explained by their increased nutritional requirements to sustain their vital functions of maintenance, growth, and lactation in comparison with the old ewes. On the other hand, ewes mated during wet season and hence lambed from September to May had shorter LI compared to those mated during the dry season. This is explained

by more forage availability during the wet season that affected positively the ewes' reproductive performance. Previous higher estimates than the current observation for LI reports in Ethiopia include 267 days for Bonga sheep (Zewdu, 2008) and 303 days for Washera sheep (Mekuriaw et al., 2013) under farmers' management. The lower LIs in the present study for the same breed could be the result of the well-structured breeding program.

5.1.3. Litter size

The overall litter size (1.43) for Bonga ewes in the present study showed wide variation (CV= 36%) indicating opportunity for genetic improvement. A higher litter size allows more selection pressure to be applied and has important indirect effect on the improvement of other important traits (Shaat et al., 2004). All parameters under consideration significantly ($p<0.001$) affected the LS of Bonga sheep (Table 2; Paper I). Similar reports for Horro sheep also indicated the significant effect of year of lambing and parity on LS (Abegaz et al., 2002). Litter size is further influenced by genotype, parity, season, and ewe body weight at mating (Mukasa-Mugarwa and Lahlou-Kassi, 1995).

In the present study, there was a general tendency for improvement in litter size as the ewe parity advanced. Likewise, reports by other authors (Mokhtari et al., 2010; Mohammadi et al., 2012) also indicated significant effect of ewe parity on litter size for different sheep breeds. There is also other evidence that season of mating influence the litter size; ewes that conceive in the dry season usually had a smaller litter size at birth than those that did it in the rainy season perhaps due to their body condition score at mating that can affect LS (José et al., 2016). Previous reports by different authors indicated varying values of LS estimates including West African sheep (1.24, Musa et al., 2005), Djallonke sheep (1.4, Gbangboche et al., 2006), and Menz sheep (1.12, Mukasa-Mugerwa

and Lahlou-Kassa, 1995). The present estimate is higher than those reported figures due to the genetic potential and other management factors of the breeding program in Bonga CBBP.

5.2. Estimates of genetic parameters

Estimates of genetic variances and heritability values are necessary for genetic evaluation of sheep to determine the selection criterion and future breeding strategies. Knowledge of genetic parameters is the basis for any sound livestock improvement programs. Due to difficulties in obtaining accurate and true genealogical information from smallholder herds, the number of studies reporting genetic parameters for reproductive traits for Ethiopian sheep breeds is generally scarce.

Estimates of heritability of a trait can vary considerably from study to study depending upon breed, population sampled, environmental and management conditions, and errors (both random and systematic) in the estimation procedures (Rosati et al., 2002). The present results confirm low heritability estimates for all traits under consideration in Bonga sheep, and these estimates were in general consistent with the estimates of other researchers. Low estimates of heritability values were mainly due to the influence of environmental factors on reproductive traits and to non-normal distributions of the traits (Dekhili, 2014). In other words, it suggests that the size of additive genetic effects are low compared to non-additive genetic effects; and thus most reproduction traits in sheep are affected by environmental factors considerably. As a result, the heritability of such traits is considerably low resulting in a slower genetic progress.

The estimate of direct heritability for LS (0.085) for Bonga sheep in the present study was in close agreement with previous reports of 0.08 by Bayeriyar et al. (2011) for Moghani Iranian sheep and 0.09 by Boujenane et al. (2013) for D'man ewes. However, the heritability estimates of LI for

Bonga ewes (0.009) was quite lower than reported figures of 0.04 by Aguirre et al. (2017) for Santa Ines sheep in Brazil. As discussed above, estimates of heritability of a trait can vary considerably from study to study depending upon breed, population sampled, environmental and management conditions, and errors (both random and systematic) in the estimation procedures. This indicates low possibility to achieve rapid genetic progress through genetic selection. With knowledge of this parameter, Bonga sheep breeders can determine whether or not a particular trait can be improved by selection, by improvement of management practices, or both.

The repeatability estimates in the present study were 0.109 and 0.196 for LI and LS, respectively, which were higher than previous observations of José et al. (2016) who reported 0.06 for LI and 0.12 for LS for Pelibuey ewes in Southeastern México. On the other hand, available reports of repeatability for LS in Ethiopia for Horro sheep (0.12) is also in close agreement with the present study (Abegaz et al., 2002). The estimates showed that the correlation between various records for the reproductive traits is low, and therefore to increase production during the lifetime of the ewes, decision about culling on one record of reproductive traits will result in low accuracy. Use of repeated model to estimate EBVs has been suggested for such traits as gain in accuracy of using repeated records is higher than using single record particularly for such traits having low repeatability (Mrode, 2014).

5.3. Estimated genetic trends

The genetic trend for litter size over the years in Bonga flocks is positive and significant. Given the low heritability of reproduction traits, genetic changes reported in the literature are, in most cases, non-significant (Haile et al., 2018). However, the current results, where litter size is one of the selection traits in both sites, indicated that positive trends could be achieved where structured

selection is implemented. Where resources like feed and water permit improvement in litter size, substantial impact in sheep production could be expected. With new genomic tools, faster genetic gains and introgression of genes into new population could be also considered. For this to happen, it is of paramount importance to investigate novel and known genomic regions affecting fertility/prolificacy in this population (Tera et al., 2019). A significant and negative trends in AFL and LI confirms that the sheep have reached their sexual maturity at younger age along with a relatively shorter interval between successive lambings as a result of selection resulting in more lambs born and hence general improvement in the flock performance.

5.4. **Follicular dynamics**

Regulation of the number of ova shed and hence litter size is crucial to successful reproduction in all mammals (Baird and Campbell, 1998). In the present study, LP and HP ewes of Bonga sheep exhibited different ovulation rates. This is supported by previous studies of Bartlewski et al. (2011), who suggested that the high ovulation rate in some prolific breeds might be achieved by the ovulation of follicles from the last two waves of the interovulatory interval. This is evidenced by the growth of antral follicles reaching ovulatory sizes that occurs in a wave-like pattern throughout the breeding season in both prolific and non-prolific breeds of sheep. McNatty et al. (2003, 2004) also suggested the oocyte controlled-processes that differed between and within species of low and high ovulation rate phenotypes and that control was mediated by oocyte-secreted factors.

In the present study, the LP ewes yielded an average litter size of 1.25 while HP ewes produced 2.12 lambs on average indicating that ovulation rate must be the upper limiting factor for sheep with low litter sizes. Results of the current study suggested that differences in ovulation rate

between the LP and HP ewes might have been caused by differences in the number of follicles growing to the large stage and the existence of co-dominance effects. In small ruminants, prolificacy is determined essentially by ovulation rate and this in turn is determined by preovulatory ovarian follicular development (Pang et al., 2010). Available reports also substantiate that higher ovulation rate observed in the HP ewe might be due to the greater number of large follicles available to be stimulated for ovulation (Bartlewski et al., 2011).

With the exception of two individuals, all the sheep displayed oestrus in a very synchronous way two days after the removal of sponges. The two exceptions, one LP and one HP had a delayed response due to individual difference on day 3 after the removal of sponges. This observation is important as it discards, at least for HP Bonga ewes, the notion that, increases in ovulation rate are related to an extended period of ovulatory follicle recruitment (Scaramuzzi and Radford, 1983). An extended period of follicle recruitment would allow a higher number of follicles growing to ovulatory stages as confirmed for various temperate sheep breeds (Souza et al., 1997; Bartlewski et al., 1999; Driancourt, 2001).

The number of small and medium follicles decreased during the follicular phase in both groups of ewes. This indicates that preovulatory follicles in both LP and HP ewes grew in a fast and continuous manner during the period of terminal growth, thereby exerting dominance over the remaining follicles. The preovulatory follicles are usually derived from the large follicle population present at the time of luteal regression. However, the sheep has the ability to promote smaller follicles if required while the second peak of FSH stimulates the development of large estrogenic follicles during the early luteal phase though the period of functional dominance is shorter than the period of morphological dominance (Carlos et al., 1997).

It has been anticipated that such a dominance effect of the preovulatory follicles to be higher in HP ewes due to the higher number of atretic follicles a day prior to oestrus (though not significant) and the lower number of new follicles during the 24 hours following oestrus. Such patterns of follicular growth are similar to those reported in other sheep breeds by various scholars (Evans et al., 2000; Gonzalez-Bulnes et al., 2001; Ben et al., 2010).

The analysed data obtained from the current study in chronological detail detail revealed some interesting trends. The mean number of CL in HP ewes is much higher than the average number of large follicles on the day of oestrus, which is in agreement with the findings of Bartlewski et al. (2011) who reported that prolific ewes tend to produce more but smaller CL. They also have lower serum concentrations of progesterone during the luteal phase of the oestrous cycle as compared to less prolific genotypes. Furthermore, while the number of medium follicles declined slightly during the follicular phase in LP ewes, it rapidly declined in HP ewes over the 48 hours preceding oestrus. Based on these two observations, it can be speculated that the medium follicles in HP ewes can be selected to ovulate, hence contributing to a higher ovulation rate that was associated with smaller follicles that contained fewer granulosa cells per thecal cell (Driancourt et al., 1996).

Throughout the follicular phase, oestradiol secretion was consistently higher in HP ewes, which is in line with previous observations of Pang et al. (2010) who reported higher overall estradiol for high prolific goats during the oestrus cycle. This is probably due to the higher number of ovulatory follicles although this does not preclude differences in functionality of the growing medium and large follicles. Such a hypothesis can only be verified with an appropriate experimental design to assess *in vitro* follicle competency.

However, it should be noted that oestradiol is also a marker for follicular function (Souza et al., 1997). As a result, preovulatory (large and medium) follicles of the HP ewes had a higher responsiveness to gonadotrophin secretion by promoting their growth prior to ovulation. What should also be noted regarding oestradiol pattern of secretion is the absence of the rise, which should normally appeared at oestrus time in both HP and LP ewes. The negative effects of progestogens on the functionality of ovulatory follicles can justify the observed phenomenon (Gonzalez-Bulnes et al., 2005).

5.5. Genetic basis of prolificacy

Genetic studies have indicated that LS and ovulation rate can be genetically determined by the action of single genes with a major effect, named fecundity genes (Vinet et al., 2012). In the present study, the F_{ST} and hapFLK identified one overlapping candidate region on OarX. As was expected, the most significant window in this region spanned BMP15 that plays a major role in various functions implicated in prolificacy (Galloway et al., 2000; Hanrahan et al., 2004). The candidate region revealed by XP-EHH on OarX was 259,479 bp downstream of BMP15. The F_{ST} test also revealed a region on Oar5 which was 4.4 Mb downstream of GDF9, an important paralog of BMP15. The BMP15 gene, that has been implicated in prolificacy in several breeds of European and Middle East sheep (Davis, 2004; 2005), occurred in this region within the most significant F_{ST} and hapFLK windows. On the other hand, a region on Oar13 that overlapped between hapFLK and XP-EHH spanned DOK5 (Docking Protein 5) gene, an adapter intracellular protein that is involved in signal transduction and is expressed in lymphocytes and T cells in human and mice and may modulate various T cell functions (Favre et al. 2003). Similar to Bonga sheep, five

different single nucleotide polymorphisms (SNPs) have been identified in the BMP15 gene (Galloway et al., 2000; Hanrahan et al., 2004) and eight SNPs in GDF9 (Hanrahan et al., 2004).

The BMP15 gene is essential for oocyte and follicular development. It is the product of an X-linked gene that is expressed in oocytes and mammals, and it helps to stimulate follicular development (Hanrahan et al., 2004). Several mutations in this gene have been identified, such as FecXI and FecXH from Romney sheep, FecXB and FecXG from Cambridge and Belclare sheep (Davis, 2005; Mullen et al, 2013), FecXL from Laucane sheep (Bodin et al., 2007), FecXR from Rasa Aragonesa, FecXGr from Grivette (Demars et al., 2013) and FecXO from Olkuska sheep (Demars et al., 2013).

As the extent of LD declines rapidly from 0 to 300 kb in the ovine genome (Kijas et al. 2014; Al-Mamun et al., 2015), LD between the SNPs found on the XP-EHH candidate region on OarX and BMP15 gene, was thus expected. This result suggests that BMP15 may be the primary candidate gene responsible for prolificacy in Bonga sheep. Experimental disruption of BMP15 in mice result in mild defects in female fertility (Su et al., 2008) whereas, natural missense mutations result in variable phenotypes in ewes, ranging from hyper prolificacy to complete sterility, depending on a fine gene dosage mechanism involving GDF9 (Belli and Shimasaki, 2018). Thus, the role of BMP15 and GDF9 in controlling ovulation rate has been formally proven in the ovine species. The BMP15 gene is also essential for oocyte and follicular development. It is the product of an X-linked gene that is expressed in oocytes in mammals, and it helps to stimulate follicular development (Hanrahan et al., 2004).

This is the first study to identify a candidate genomic region and gene associated with prolificacy in an indigenous Sub Sahara African (SSA) sheep breed; it is, therefore, possible that BMP15 will encode the trait in other prolific SSA sheep. It would be of interest to investigate whether the

causative variant(s) are the same or novel across prolific SSA sheep breeds *vis-à-vis* the ones found in Europe and Middle East. This will shed light on the evolution of the genetic basis of the trait and is of relevance since at least 8 mutations have been reported, so far, in BMP15 while a new variant was recently found in the Barbarine sheep (Lassoued et al., 2017) and three Iranian sheep breeds (Amini et al., 2018).

A genomic work has led to the identification of a mutation linked to BMP15 as the main candidate in Bonga sheep (Tera et al., 2019). Thus, the role of BMP15 and GDF9 in controlling ovulation rate has been formally proven in the ovine species. Physiologically this study provides evidence characterizing high litter size in Bonga sheep mainly reflected in the growth of larger preovulatory follicles and in the existence of co-dominance effects (Tera et al., 2020) as higher number and smaller diameter characterize the ovulatory follicles of prolific breeds. Thus, the fate of follicles depend on the balance between stimulatory and inhibitory factors. This mainly acts in one of the three mechanisms as increased activation of an augmenter, decreased activation of attenuators or combination of these two mechanisms. These two genes are expressed in oocytes and have been shown to be essential for ovulation rate, normal follicular growth and maturation of preovulatory follicles (McNatty et al., 2004). Appropriate design of breeding programmes is therefore impossible for breeds that have not been adequately characterized either phenotypically and/or genetically (Mwacharo et al., 2006).

The genes linked to immune functions that are overrepresented in this study responsible for immune system might have protected the Bonga sheep living in humid and moist climate where there are many types of diseases like *Haemoncus contortus* (Yang et al., 2015). In agreement with this Mwacharo et al. (2017) also reported over-representation of immune related genes in the genomes of tropically adapted livestock. Like in other mammals an ejaculated spermatozoon is

normally retained in functional pre-ovulatory sperm reservoirs in female genital tracts until ovulation. Rather than being eliminated, the immunologically foreign sperm is tolerated by the female immune defence system. It has been observed that semen can signal genomic shifts that modulate gene expression of genes linked to immune function resulting in a state of immune tolerance during the lengthy storage of spermatozoa in oviductal sperm reservoirs (Holt and Fazeli, 2016). It is, therefore, a possibility that high prolificacy has made the oviduct of prolific individuals less responsive to antigenic seminal fluid. This creates an appropriate immune-balanced physiological environment tailored for sperm survival and fertilization.

What is most interesting and novel in this study not reported previously in any prolific sheep, but has been associated with female and male reproduction traits in other species, is the identification of novel candidate genes. The ones reported in female animals are *SPOCK1, PKD2L2*, *HB-EGF*, *GPR173, MAGED1*, *SMARCAL1*, *HMGN3a, ELK3*, and *KDM3B*. *SPOCK1,* which marks the beginning of a females reproductive life (Dvornyk and Waqar-ul-Haq, 2012), in beef cattle (Fortes et al., 2010) and humans (Liu et al., 2009). The candidate regions identified in this study also spanned several genes implicated in male fertility and reproduction in other species, but not reported in prolific sheep. They included *FOXJ1*, *NME5, PKD2L2*, *MAGED1* and *KDM3B* in which the latter three genes have been implicated in female reproduction. The occurrence of these genes in the candidate regions suggest that they are essential in the maintenance of the processes of spermatogenesis and normal male sexual behavior to ensure successful fertilization of a large number of ova generated in prolific ewes.

Common strategy for increasing prolificacy via genetic means is to select ewes that are more likely to produce multiple births and to select rams that are more likely to sire prolific daughters. However, genetic improvement programs for small ruminants are scarce due to lack of

performance and pedigree information and the non-existence of institutional frameworks and infrastructure including inadequate farmers' organizations at the community level to effectively participate on breeding schemes (Kosgey and Okeyo, 2007). However, this has been in place in Ethiopia for the last ten years in Community-Based Bonga sheep breeding program that is the one under operation.

Genome wide studies in Bonga sheep revealed major genes for prolificacy with differing sizes of effect on ovulation rate and litter size. Apart from these genes responsible for immune system, male and female fertility traits have been identified and have become a new option for sheep farmers aiming to significantly increase lambing percentages (Davis, 2005). Thus, the incorporation of a major gene for prolificacy into a flock using marker-assisted selection allows increased selection pressure on other traits leading to increased genetic gain. A major gene has the advantage that it can be introduced into any new breed while retaining the new breed's characteristics.

6. SUMMARY AND CONCLUSION

6.1. Summary

With a large and diverse population of 33 million, sheep production in Ethiopia contributes substantially to the livelihood and income of the rural community and the country at large. However, the absence of appropriate breeding programmes has long been one of the reasons for low productivity. Hence, genetic improvement could be one of the means to bridging the productivity gap and contribute to reversing the challenges the sector faces. The knowledge of genes that are involved in the reproductive traits and the effects they have provides useful information for breeding and selection on those target traits. In this regard, the Bonga sheep is a breed of high reproductive potential and good maternal abilities. Therefore, maintaining its overall reproductive performances is of a major importance. This study was undertaken with three major objectives: to evaluate the effects of non-genetic factors on reproductive traits like age at first lambing (AFL), lambing interval (LI) and litter size (LS) thereby estimating the genetic parameters and trends; to characterize physiological and endocrine basis of LS; and to identify the genetic basis of prolificacy.

Ten years data on reproductive traits of Bonga sheep were analyzed by fitting breeding communities, season of mating, year of lambing, and parity as fixed effects. The genetic parameters and breeding values of these traits were also analyzed using Restricted Maximum likelihood procedure of WOMBAT by fitting the univariate animal model. Based on LS records, 31 ewes were selected and further divided into two groups of high and low prolificacy. At a synchronized oestrus, follicular size and counts were determined using transrectal ultrasonography

while plasma oestradiol concentrations were monitored throughout the induced follicular phase. Whole blood was collected from 95 ewes that gave birth of singles, twins, triplets, and quadruplets for genome-wide analysis. Candidate regions under selection were identified using selection signature analysis performed on Ovine HD BeadChip data.

Non-genetic factors including breeding communities, lambing year, mating seasons and ewe parity had significant effect on AFL, LI and LS of Bonga ewes. Heritability estimates of these traits in Bonga sheep were rather low and thus low possibility to achieve rapid genetic progress through phenotypic selection and thus inclusion of genetic information is important. Repeatability estimates for LI and LS were low indicating that environmental factors contributed appreciably to the variation in these traits among parities and hence ewes should not be culled on a single or few initially available phenotypic records. The genetic trend for AFL, LI and LS over the years were all significant implying that the breeding program implemented with the communities over 10 years has resulted in measurable genetic gains for reproductive traits.

The ovarian level of the phenotypic variability in LS were mainly reflected in the growth of large number with small diameter of pre-ovulatory follicles growing to the large stage and the existence of co-dominance. The lambing data consecutive to the following protocol is consistent with the hypothesis as low prolific (LP) ewes yielded an average litter size of 1.25 while high prolific (HP) ewes produced 2.12 lambs on average indicating that difference in ovulation rate must be the upper limiting factor for difference in LS between HP and LP Bonga ewes.

Identification of genes associated with prolificacy in Bonga sheep, revealed several known and novel candidate genes implicated in male and female fertility, reproduction and immunity in other species, suggesting that such genes could be hotspots of selection in indigenous Sub-Saharan Africa prolific breeds of sheep. The findings further suggested that enhanced reproduction in

prolific ewes entails not only prolificacy genes but also epistatic effects with genes associated with other reproduction traits. Moreover, BMP15 was identified as the main candidate gene for prolificacy (with its companion GDF9) in Bonga sheep.

6.2. Conclusion

Although reproductive traits have low to medium heritability and thus do not exhibit a noticeable response to phenotypic selection in traditional breeding methods, measurable improvements have been obtained in the structured Bonga CBBP. Any selection decision for the traits having repeated measurements (LI and LS) need to be based on repeated animal model to maximize accuracy in the estimation of breeding values besides considering the non-genetic factors. Increased number of large preovulatory follicles and the existence of co-dominance effects contributed to the differences in ovulation rate and subsequent litter size between low and high prolific Bonga ewes. The mechanism of action for novel and major genes BMP15 and GDF9 identified in Bonga sheep revealed the existence of mutation. Thus, the use of such novel genes in Bonga sheep CBBP via genomic selection, marker-assisted selection, or genome-wide association studies could allow increased selection pressure leading to increased genetic gain of reproductive performance traits. Moreover, such major genes can be introduced into any new breed where applicable while retaining the new breed's characteristics giving new option for sheep farmers aiming to increase lambing percentage.

7. SCOPE FOR FUTURE WORK

1. Although BMP15 was identified as the main candidate gene for prolificacy in Bonga sheep, the exact causative variants need to be determined to further confirm whether they are novel or are part of what has been reported elsewhere in prolific breeds of sheep from Europe and the Middle East.
2. The incorporation of the genetic information, such as revealed here, in such breeding programmes (e.g. CBBPs) via either genomic selection, marker-assisted selection, or genome-wide association studies could enhance response to selection towards the genetic improvement of reproductive performance.

8. LIMITATIONS OF THE STUDY

1. It is important to note that the sample size (84 individuals) used for genotyping study is relatively low. This may have underpowered the genome analysis; thus, the present findings should be interpreted with caution and need validation using a larger subset of animals and populations.
2. The same individual animals should have been used for genotyping once the follicular and endocrine changes study was completed to better confirm the current findings; however, as these animals are managed by farmers, they could not be easily available for the intedned study.
3. Bonga ewes that are sterile couldn't be also available to confirm the current findings.
4. Data collected at farm level lacks consistency and accuracy and hence many phenotypic observations were rejected during data handling and cleaning.

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10. INDIVIDUAL PAPERS

Paper I

Estimates of genetic parameters and trends for reproduction traits in Bonga sheep, Ethiopia

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REGULAR ARTICLES

Estimates of genetic parameters and trends for reproduction traits in Bonga sheep, Ethiopia

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Abstract
Investigation was carried out to assess the effects of environmental factors and to estimate genetic parameters and trends for reproductive traits in Bonga sheep, Ethiopia. Animals used in this study were managed by two communities involved in a community-based breeding programs (CBBPs) from 2009 to 2018. The database consisted of 15,595 individual phenotypic information from about 1500 ewes with variable number of records for each trait. The traits analyzed were age at first lambing (AFL), lambing interval (LI), and litter size (LS). Fixed effect analysis was done using the general linear model procedures of SAS. The Average Information Restricted Maximum Likelihood method of WOMBAT, fitting univariate animalmodel, was used to estimate heritabilities, repeatabilities and breeding values. Results showed that Bonga sheep had overall mean AFL, LI, and LS of 453 ± 109 days, 254 ± 51 days, and 1.43 ± 0.008, respectively. All traits were influenced ($p < 0.01$) by lambing year, lambing season, breeder communities, and parity of ewes. Estimates of heritability for AFL, LI, and LS were 0.015 ± 0.143, 0.009 ± 0.070, and 0.085 ± 0.110, respectively. The low heritabilities for the traits are expected and indicate low possibility of achieving rapid genetic progress through phenotypic selection. The repeatability estimates for LI and LS were low (0.109 and 0.196, respectively) indicating that non-genetic factors had significant influence to the variation in these traits among parities; therefore, selection decision on ewes should consider repeated records. The genetic trend for AFL, LI, and LS over the years was significant ($p < 0.01$). Positive values for LS and negative for AFL and LI were recorded, implying that the well-structured CBBPs have resulted in measurable genetic gains for the reproductive traits.

Keywords Age at first lambing · Lambing interval · Litter size · Heritability · Breeding values

Introduction

Community-based breeding programs (CBBPs) in small ruminants have been suggested as attractive and sustainable genetic improvement options in low-input systems (Haile et al. 2019). Such breeding programs which consider community needs and views have been designed and implemented in Bonga, Ethiopia, since 2009. In CBBPs, the core implementation procedures include defining breeding objectives and goal traits in a participatory way, designing breeding structures suitable for different production systems, data recording using enumerators on identified traits, selection of breeding sires based on estimated breeding values, and communal use of these sires (Haile et al. 2018). In Bonga CBBPs, the most important reproductive performance traits identified by the communities on which data is being recorded included age at first lambing (AFL), lambing interval (LI), and litter size (LS).

These traits are very important in sheep production because reproductive rate can be increased in sheep by reducing AFL, increasing number of lambs per lambing, reducing number of days between lambings, and reducing lamb mortality. Age at first lambing influences lifetime reproduction and is recommended for inclusion in the selection program in sheep (Schoeman and Jaeneltte 1991). Ewes lambing for the first time

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at 1 year of age allow producers to cull infertile animals (Kutluca and Emsen 2016). Higher LS allows more selection pressure; differences in LS contribute more to total lamb weight weaned per ewe than growth rate of individual lambs (Shaat et al. 2004).

Accurate estimates of genetic parameters are essential to design efficient genetic improvement programs and are best tools available to maximize the response to a selection program (Safari et al. 2005). Estimates of genetic parameters are also necessary to determine the selection method to be used, to estimate the maximum genetic gain achieved, and to obtain correct estimates of breeding values. However, studies on genetic effects for reproductive traits in sheep are scarce in tropical areas due to the lack of genealogical data recording in extensive production systems. The paper is based on data collected from CBBPs in Bonga with the hypothesis that genetic progress for reproductive traits could be achieved in structured breeding programs and has the following objectives: (1) to investigate the effects of non-genetic factors on reproductive traits of Bonga sheep, (2) to estimate (co)variance components and genetic parameters for some reproductive traits of Bonga sheep, and (3) to assess genetic progress for reproductive traits in Bonga sheep.

Materials and methods

The study area and the breed

The data was obtained from the database of an ongoing CBBP for Bonga sheep which is being implemented in two communities (Boka and Shuta) of the Kafa zone of Southern Nations, Nationalities, and People's Region of Ethiopia since 2009. Boka-Shutta is located at 509 km south west of Addis Ababa. It has wet humid agro-ecology with an average altitude of 2511 m asl, 7° 17′ N latitude and 36° 24′ E longitude, and mean annual temperature ranges of 17.5–22.5 °C. The area is covered by large ever green natural forest receiving year-round rain fall. The prominent farming system in the area is mixed crop–livestock production.

The Bonga sheep breed is characterized by a wide and moderately long tail; both males and females are mostly polled and have long ears and short and smooth hair (Edea et al. 2009). The breed is judged good for traits such as growth rate, meat quality, fattening potential, twinning rate, and temperament (Edea et al. 2009).

Breeding scheme and animal management

The Bonga CBBP is currently being implemented jointly by the Bonga Agricultural Research Center (BARC) and the International Center for Agricultural Research in the Dry Areas (ICARDA). Breeding objectives as defined by the community were to improve growth, twining rate, and mothering ability (lamb survival) of Bonga sheep. Recording of lambing and growth performances was routinely done in the CBBP. An enumerator was recruited for the CBBP and is responsible for data collection and recording. Data validation and entering into the computer have been implemented by BARC breeders. Average flock size per household was about 9.7. A mating group usually comprised of 25 to 30 breeding ewes. Breeding sire has been selected at 6 months of age based on estimated breeding values of farmer's selection traits. Ewes were culled if they had health or reproductive problems. All animals in the database were managed by farmers. Flocks remain tethered in pastures throughout the daytime and penned during night. Ewe/ram lambs were housed until 20 days from the day of birth and then released onwards with their dams freely for grazing. Feed is abundantly found in the area, and sheep are mainly grazed on *Cynodon dactylon* and browse *Brugmansia suaveolens* Bercht. Predominantly controlled mating was practiced where ewes in heat were brought to selected and allocated ram. Detailed description of the breeding program is given in Haile et al. (2018).

Data management

The database consisted of 15,595 individual phenotypic information from about 1500 ewes with variable number of records for each trait (Table 1). Pedigree information was only partially complete, and a pedigree viewer software was used to check for errors in duplicate animals, biscxuality, sire of itself, and dam of itself errors in the data set. Screening outliers was employed before conducting the main data analysis. Factors considered fixed environmental effects in the model included lambing season: wet season from April to December and dry season from November to March, ewe parity (1, 2, 3, 4, 5, 6, 7, and ≥ 8), year of lambing (2009–2018), and CBBP communities (Boka and Shuta). Traits analyzed in the present study were AFL, LI, and LS at birth. Age at first lambing and LI were calculated from the recorded information.

Data analysis

The significance of fixed environmental effects was tested by least squares analyses of variance using the general linear

Table 1 Number of records and pedigree structure for age at first lambing (AFL), lambing interval (LI), and litter size (LS) of Bonga sheep kept under a community-based breeding program during 2009 to 2018

	AFL	LI	LS
Number of records	1142	3009	8590
Number of sires	84	101	58
Number of dams	121	236	140

model (GLM) procedures of the Statistical Analysis System (SAS Institute Inc 2009).

The model used for analysis of AFL was $y_{ijkl} = \mu + S_i + A_j + E_k +$ eijk, where y_{ijkl} = observations corresponding to AFL; μ = overall mean; S_i = effect of communities (Boka and Shuta); A_j = effect of year of lambing (2009–2018); E_k = effect of season of lambing (wet and dry); and eijk = residual effect, normal, and independently distributed.

For LS and LI, the model was:

$y_{ijkl} = \mu + S_i + A_j + E_k + N_l +$ eijkl, where y_{ijkl} = observations corresponding to LS and LI; μ = overall mean; S_i = effect of communities (Boka and Shuta); A_j = effect of year of lambing (2009–2018); E_k = effect of lambing season (wet and dry); N_l = effect of parity of ewe (1, 2, 3, 4, 5, 6, 7, and ≥ 8); and eijkl = residual effect, normal, and independently distributed.

Genetic (heritability and repeatability) and phenotypic parameters were estimated using the Restricted Maximum Likelihood (REML) algorithm of the WOMBAT software (Meyer 2007). A univariate animal model including maternal genetic and permanent environmental maternal effects as additional random effects was fitted for all data sets. Heritability (h^2) was calculated as the ratio of animal variance to phenotypic variance. Repeatability for LI and LS was estimated by dividing the variance components (animal and permanent environmental) of ewe to the phenotypic variance.

Model fitted was $Y = \boldsymbol{X}b + \boldsymbol{Z}a + \boldsymbol{W}\mathrm{pe} + e$

where

y	vector of observation for each trait,
b	vector of fixed effects,
a	vector of random animal effects,
pe	vector of random permanent environment effect of ewe,
e	vector of random residual effects,
$\boldsymbol{X}$, $\boldsymbol{Z}$, and $\boldsymbol{W}$	are incidence matrices relating to records of fixed, animal, and permanent environmental effects, respectively.

The genetic trend was estimated by the weighted regression of the average breeding value of the animals on the year of birth/lambing.

Results

Fixed effects

The effects of fixed factors of breeding communities (sites), lambing year, lambing season, and parity of the ewe on all reproductive traits were highly significant ($p < 0.001$) except for the effect of site on AFL which was not significant (Table 2). LI was shorter and LS higher in Boka than Shuta. Dams of Boka gave birth to lambs nearly 8 days earlier (251 ± 1.24) than that of Shuta (259 ± 1.36). Furthermore, dams of Boka gave more lambs (1.47 ± 0.009) than Shuta (1.39 ± 0.009) ewes. Although Boka ewes also gave birth to lambs at slightly younger age (453 ± 6) than Shuta (461 ± 6) ewes, the difference was not significant (Table 2).

Lambing year had significant effect on traits analyzed with wide fluctuations from year to year. No clear pattern could be established regarding the variation for any of the three traits analyzed because of their inconsistency across the years. Lowest LI (245 ± 2.10 days) was observed in 2015, whereas the highest one (269 ± 5.7 days) was in 2018. There was a decreasing trend in LI till 2015 from the onset of selection with very slight pick in 2013, but it seemed increasing in 2018. Similarly, LS had no regular pattern across the lambing years, but the lowest LS was observed in ewes that lambed in 2014 and 2017. At the study sites, months from April to December are wet seasons where there is ample amount of feed. Ewes that were mated from April to December (hence lambed during September to May) had shorter LI (245 ± 1.18) than those mated in dry season, from November to March (lambed during April to August), which was 264 ± 1.6 days. Similarly, ewes mated in wet season had higher number of lambs (1.46 ± 0.009) than those mated in dry season (1.39 ± 0.009). Ewes which were mated in wet season also had shorter AFL (443 ± 5) than those mated in dry season (471 ± 8). Ewe parity showed significant effect on the traits investigated but did not follow a regular pattern. There has been improving trend in LI as parity advanced from parity 2 to parity 4 and above. On the other hand, the order of ewe parity had pronounced effect on the number of lambs born per ewe with the highest litter size at parity six.

Genetic parameters

Estimates of (co)variance components, heritability, and repeatability of the studied traits are presented in Table 3. The direct animal variance components for AFL, LI, and LS were 176.1, 21.2, and 0.020738, respectively. On the other hand, the phenotypic variance is 11,425 for AFL, 2394.7 for LI, and 0.24395 for LS. As a result, the heritability estimates with their respective standard errors for AFL, LI, and LS for Bonga sheep were 0.015 ± 0.143, 0.009 ± 0.070, and 0.085 ± 0.110, respectively. The repeatability estimates were 0.109 and 0.196 for LI and LS, respectively. The number of lambs born had relatively higher heritability and repeatability than the other traits under investigation.

Genetic trends

The genetic trend for LI, AFL, and LS over the years is significant ($p < 0.01$; Figs. 1, 2, and 3). While the trend is positive

Table 2 Least squares mean (LSM) ± standard error (SE) of age at first lambing (AFL), lambing interval (LI), and litter size (LS) of Bonga sheep

Factor and level	LI (days)		LS (number)		AFL (days)	
Overall	*N*	LSM ± SE	*N*	LSM ± SE	*N*	LSM ± SE
	2993	253.5 ± 0.92	8590	1.43 ± 0.008	551	453 ± 4.65
	CV (%)	19.2		36		23.5
	N	LSM ± SE	*N*	LSM ± SE	*N*	LSM ± SE
Site		***		***		NS
Boka	1573	251 ± 1.24	4433	1.48 ± 0.009	278	453 ± 6
Shuta	1420	259 ± 1.36	4157	1.39 ± 0.010	273	461 ± 6
Lambing Year		***		***		**
Mating season		***		***		**
Wet season	1683	245 ± 1.18	4815	1.46 ± 0.010	194	443 ± 5
Dry season	1310	264 ± 1.40	3775	1.41 ± 0.009	357	471 ± 8
Parity		***		***		
1			2169	1.26 ± 0.011^{f}		
2	652	269 ± 2.00^{a}	1698	1.37 ± 0.013^{e}		
3	568	261 ± 2.22^{b}	1288	1.40 ± 0.015^{de}		
4	499	254 ± 2.24^{c}	1108	1.44 ± 0.016^{cd}		
5	401	249 ± 2.32^{c}	800	1.47 ± 0.019^{bc}		
6	325	249 ± 2.41^{c}	600	1.54 ± 0.021^{a}		
7	208	253 ± 3.36^{c}	370	1.48 ± 0.027^{ab}		
8 and above	340	248 ± 2.84^{c}	557	1.51 ± 0.022^{ab}		

Least squares mean within the same column in the same category with different superscript is significantly different

***< 0.001

**< 0.01

*< 0.05

NS, non-significant; *N*, number of observation

for LS, understandably, there is a decreasing trend for AFL and LI.

Discussion

Fixed effects

The improvement of environmental conditions (management and nutrition) and the use of genetic selection were considered two main ways for improving reproductive efficiency in sheep. Whatever environment is considered, reproductive and survival traits in small ruminant production systems are undoubtedly the most important ones (Matika et al. 2003). Coefficient of variation for a particular trait is a criterion to determine the trait variation. Coefficient of variation for the studied traits was 19.2% for LI, 36% for LS, and 23.9% for AFL. Reports of CV for LS for different breeds of sheep available include 30.5% for Sabi sheep (Matika et al. 2003), 37.3% for Santa Ines sheep of Brazil (Aguirre et al. 2017), and 30.23% for Zandi sheep of Iran (Mohammadi et al. 2012). In contrast, lower CV (11.6%) for AFL and 28.7% for LI for Santa Ines sheep of Brazil (Aguirre et al. 2017) were also reported. The variabilities existing in flock are indicative of

Table 3 Estimates of genetic parameters for age at first lambing, lambing interval, and litter size in Bonga sheep during 2009 to 2018

Trait	σ_a^2	σ_{pe}^2	σ_{res}^2	σ_p^2	$h_d^2 \pm$ SE	$Pe^2 \pm$ SE	*r*
AFL	176.1	-	11,249	11,425	0.015 ± 0.143	-	-
LI	21.2	240.8	2132.7	2394.7	0.009 ± 0.070	0.101 ± 0.072	0.109
LS	0.020738	0.027001	0.19621	0.24395	0.085 ± 0.110	0.111 ± 0.111	0.196

σ_a^2 = Direct additive variance, σ_{pe}^2 = permanent environmental variance, σ_{res}^2 = residual variance, σ_p^2 = phenotypic variance, h_d^2 = direct heritability, Pe^2 = ratio of permanent environment variance on phenotypic variance, *r* = repeatability, SE = standard error

AFL, age at first lambing (in days); *LI*, lambing interval (in days); *LS*, litter size

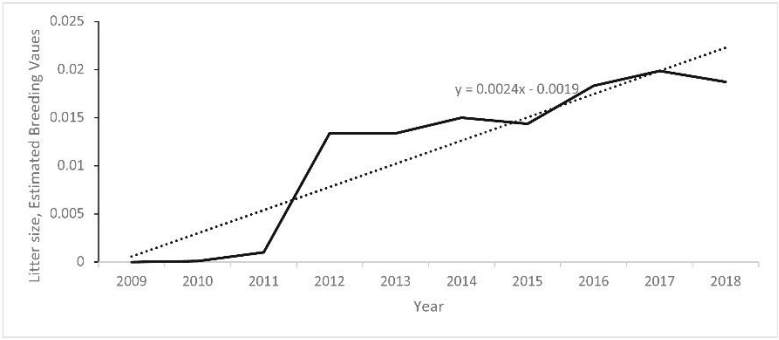

Fig. 1 Genetic trends for litter size for Bonga ewes in community-based breeding programs during 2009 to 2018

the genetic improvement that could be made through selective breeding.

The LI, LS, and AFL in the present study were affected by year of lambing of the ewe; although means between years did not show any clear pattern. Effects of CBBP communities (sites), lambing year, mating season, and ewe parity in the present study were statistically significant ($p < 0.001$) for all studied reproductive traits. In agreement with our findings, Berhanu and Aynalem (2009) reported significant effect of season of lambing and parity of the ewe on LS for Ethiopian sheep in south western Ethiopia. Various workers have revealed the significant effect of year and lambing season in Awassi sheep breed of Syria (Mavrogenis 1996) and parity for D'man ewes of Morocco (Boujenane et al. 2013) for LI, LS, and AFL.

From the two communities where CBBPs have been operating in Bonga sheep, the Boka site was better than Shuta for all reproductive traits. The two sites are located about 10 km apart, and they have no major nutritional or environmental differences. In fact, they are genetically related to 6 rams used in the two flocks. Therefore, the difference could be associated with level of commitment of CBBP farmers and enumerators, which lead to difference in accuracy of data collected.

The overall litter size (1.43 ± 0.008) for Bonga ewes in the present study has wide variation (CV = 36%). Previous reports by different authors indicated varying values of LS estimates including for West African sheep (1.24, Musa et al. 2005), Djallonke sheep (1.4, Gbangboche et al. 2006), and Menz sheep (1.12, Mukasa-Mugerwa and Lahlou-Kassi 1995). In the present study, there was a general tendency for improvement in litter size as the ewe parity advanced. Likewise, reports by other authors (Rosati et al. 2002; Vatankhah et al. 2008; Mokhtari et al. 2010; Mohammadi et al. 2012) also indicated significant effect of ewe parity on litter size for different sheep breeds. There is evidence that seasonal factors influence the litter size; ewes that conceive in the dry season usually have a smaller litter size at birth than those that do it in the rainy season, perhaps due to their body condition score at mating that can affect LS (José et al. 2016).

Age at first lambing of African sheep has wide variation due to breed, environmental, and other factors. The average AFL of Bonga ewes managed at farmers' level in the present study was estimated at 453 ± 10.26 days. This result seems a bit higher than the expected figure, probably due to the small data size. In contrast to this report, Berhanu and Aynalem (2009) reported the mean AFL as 404 days in Ethiopian sheep

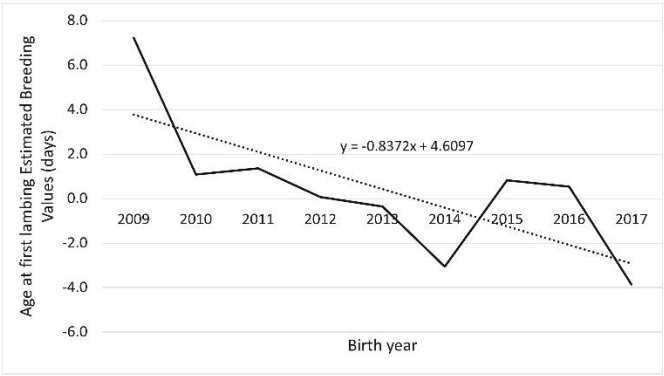

Fig. 2 Genetic trends for age at first lambing for Bonga ewes in community-based breeding programs during 2009 to 2018

which is lower than our report. In a separate report, Demeke et al. (1995) indicated AFL of Ethiopian highland sheep as 471 ± 6 days. On the other hand, José et al. (2016) reported as high as 526.7 ± 107.1 days for Pelibuey ewes in Southeastern México. Yadav et al. (2018) also estimated AFL for Munjal Sheep at 713.48 ± 14.02 days. Such a wide variability in AFL between different breeds could be attributed to both genetic difference and most importantly to difference in the management system followed.

The LI for Bonga sheep under the present study is 254 days, which is significantly ($p < 0.0001$) affected by breeding communities, lambing year, mating season, and ewe parity. Previous report by Mukasa-Mugerwa and Lahlou-Kassi (1995) indicated LI for Menz sheep as 252 days. In contrast to this report, lower days of LI reported include for West African sheep breed (207 days, Musa et al. 2005) and Djallonke sheep (242.4 days, Gbangboche et al. 2006). Higher LI estimates for different breeds have also been reported: 259.4 days for Pelibuey ewes in Southeastern México (José et al. 2016), 306 days for Iranian Afshari flocks (Mohammadi et al. 2012).

Lambing interval is affected by the breed, year/season of lambing, parity of ewes, and type of management (Gbangboche et al. 2006). The significant influence of lambing year in the present study can be attributed to variation in the climatic conditions leading to variation in pasture availability, difference in management and breeding conditions of ewes, and lamb feeding in different years that cause fluctuations in reproduction performance (Kumar et al. 2017). Significant effects of lambing year on reproductive performances have been reported by several authors (Mavrogenis 1996; Mohammadi et al. 2012). Similarly, mating season has also shown significant influence ($p < 0.001$) in reproductive traits explained by more forage in long wet season which positively affects the ewes' reproductive performance.

Genetic parameter estimate

Estimates of genetic parameters are important to determine the selection criterion and future breeding strategies. Due to difficulties in getting accurate and true genealogical information in smallholder herds, number of studies reporting genetic parameters for reproductive traits for Ethiopian sheep breeds is minimal. The present results confirm low influence of genetic assumptions on LS. The estimate of direct heritability for LS (0.085 ± 0.110) for Bonga sheep was in close agreement with previous reports of 0.08 ± 0.01 by Bayeriyar et al. (2011) for Moghani Iranian sheep and 0.09 ± 0.04 by Boujenane et al. (2013) for D'man ewes. However, our result is lower than the estimates reported by various workers, 0.17 (Mavrogenis 1996) for Awassi sheep and 0.14 (Mohammadi et al. 2012) for Zandi sheep of Iran. Lower estimates have also been reported, for example, 0.05 (van Wyk et al. 2003), 0.06 (Maxa et al. 2007), and 0.01 (Mokhtari et al. 2010). The heritability estimates for AFL for Bonga ewes (0.015 ± 0.143) are lower than the reported values of 0.17 ± 0.10 for Munjal sheep of India (Yadav et al. 2018) and 0.13 for Santa Ines sheep of Brazil (Aguirre et al. 2017).

The heritability estimates of LI for Bonga ewes (0.009 ± 0.07) were quite lower than reported figures of 0.04 ± 0.017 (Aguirre et al. 2016) for Santa Ines sheep in Brazil. Estimates of heritability of a trait can vary considerably from study to study depending upon breed, population sampled, environmental and management conditions, and errors (both random and systematic) in the estimation procedures.

Most reproduction traits, including AFL, LI, and LS, have low heritability estimates. In other words, additive genetic effects have little effect on reproduction traits, while environmental and non-additive genetic effects considerably affect these traits. Low heritability indicates low possibility to achieve rapid genetic progress through phenotypic selection.

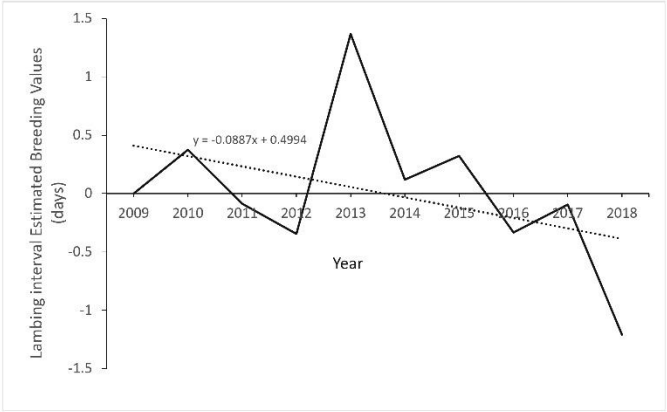

Fig. 3 Genetic trends for lambing interval for Bonga ewes in community-based breeding programs during 2009 to 2018

The repeatability estimates in the present study for Bonga sheep (0.109 and 0.196 for LI and LS, respectively) were higher than previous reports by José et al. (2016) who reported 0.06 for LI and 0.12 for LS for Pelibuey ewes in Southeastern México. The estimates show that the correlation between various records for the reproductive traits is low, indicating that non-genetic factors had significant influence to the variation in these traits among parities; therefore, selection decision on ewes should consider repeated records.

Genetic trends

The genetic trend for litter size over the years in Bonga flocks is positive and significant. Given the low heritability of reproduction traits, genetic changes reported in literature are, in most cases, non-significant (Abdoli et al. 2019). However, our results, where litter size is one of the selection traits in both sites, indicate that positive trends could be achieved where structured selection is implemented. Where resources like feed and water permit improvement in litter size, substantial impact in sheep production could be expected. With new genomic tools, faster genetic gains and introgression of genes into new population could be done. For this to happen, it is of paramount importance to investigate novel and known genomic regions affecting fertility/prolificacy in this population. Similarly, the genetic trends for AFL and LI are also significant and negative indicating that sheep that reached reproductive age at younger age and relatively smaller days between successive lambing were achieved as selection progressed, resulting in more lambs born and hence general improvement in the flock performance.

In conclusion, development of breeding strategies and effective genetic improvement program requires knowledge of the genetic variation among important traits and accurate estimates of genetic parameters. Results showed that Bonga sheep compares reasonably with other tropically adapted populations for reproductive performances. Heritability estimates for the reproductive traits, like in other reports, are low and indicate low possibility of achieving rapid genetic progress through phenotypic selection even though we have achieved genetic gains in our structured CBBPs. The repeatability estimates for LI and LS were also low, indicating that non-genetic factors had significant influence to the variation in these traits among parities; therefore, selection decision on ewes should consider repeated records. The genetic trends for AFL, LI, and LS over the years were all significant, implying that the breeding program implemented with the communities has resulted in measurable genetic gains for the reproductive traits.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Paper II

Increased number of large non-atretic follicles and co-dominance effects account for high litter sizes in Bonga sheep

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ORIGINAL ARTICLE

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Increased number of large non-atretic follicles and co-dominance effects account for high litter sizes in Bonga sheep

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Abstract

To understand the ovarian basis for prolificacy of Bonga sheep, a total of 31 ewes were selected based on litter size (LS) records and divided into two groups: High Prolificacy (HP) (n = 20) with LS ≥ 2 and Low Prolificacy (LP) (n = 11) with LS = 1. At a synchronized estrus, follicular dynamics were determined using transrectal ultrasonography. Plasma estradiol concentrations were also monitored. In total 27 ewes were observed in estrus being 9/11 LP (82%) and 18/20 HP (90%). On the day of estrus (day 0), the mean number of large follicles was higher ($p < .05$) in HP (1.78 ± 0.19) than in LP (1.0 ± 0.28) ewes. Prior to estrus, more ($p < .05$) medium follicles were visible for HP compared to LP ewes. Plasma estradiol concentrations were higher in HP compared to LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; $p < .05$) and similarly was ovulation number (2.3 ± 0.15 vs. 1.28 ± 0. 14; $p < .05$). Higher ovulation rates and litter size in Bonga sheep are evidenced by the previous presence of more large follicles and the existence of co-dominance effects as most likely medium follicles are selected to ovulate.

KEYWORDS
Bonga sheep, estradiol, follicles, litter size, ovulation rate

1 | INTRODUCTION

Sheep population in Ethiopia is among the largest in East Africa and sub-Saharan Africa and it increased from 25.5 million in 2012 to 31.8 million in 2017 (FAOSTAT, 2017). Small ruminants are mainly kept by smallholder farmers and the rural poor. Sheep contribute substantially as a source of income, food (meat and milk), and industrial raw materials (skin and wool). In addition, sheep production has a socioeconomic and cultural function and contributes to risk mitigation during crop failures, increase property security, and serve as a form of investment (Tibbo, 2006).

Ethiopia has a large and diverse sheep population which is divided to four groups based on morphological characteristics and geographic distribution: (a) Sub-Alpine short-fat-tailed, (b) Highland long-fat-tailed, (c) Lowland fat-rumped/tailed, and (d) Lowland thin-tailed groups (Gizaw, Komen, Hanotte, & Van Arendonk, 2008). Bonga sheep belong to the Highland long-fat-tailed group and have physical features characterized by long fat tail with straight tapering end (98.4%), hairy coat, large size, and predominantly plain brown in color (57.9%; Gizaw et al., 2011). Average adult body weights of female and male Bonga sheep are 32 and 48 kg, respectively (Edea, 2008), and the breed is considered among the large-sized sheep breeds in Ethiopia. Kaffa, Sheka, and Bench zones of Southern State are the home region of the Bonga sheep with 66% of the total population being reared in Kaffa zone (CSA, 2017).

The reproductive performance of sheep in Ethiopia varies among breeds, types, and locations. Major traits differences include age at first lambing, litter size, lambing interval, and lifetime productivity of the ewe (Abate, 2016).

In a study carried out in Adiyo Kaka (Southern Nations, Nationalities and Peoples' Regional State of Ethiopia) district, Edea et al. (2012) calculated an average age at first lambing of 14.9 ± 3.12 (±*SE*) months for Bonga sheep which is younger than the age reported for Menz sheep (523 ± 13 days) in the Ethiopian highlands (Mukasa-Mugenva et al., 1991). Litter size is directly related to ovulation rate, genotype, and environmental factors (Mukasa-Mugerwa & Lahlou-Kassi, 1995). A litter size of 1.36 was reported for Bonga sheep (Edea et al., 2012) which was higher than the 1.14 reported for Menz sheep under traditional village conditions (Agyemang, Negussie, Voorthuize, & Anderson, 1985). Average lambing interval for Bonga breed is 8.92 ± 2.13 months (Edea et al., 2012) being shorter than the 10 to 11 months figure for Menz sheep (Dibissa, 1990). On average, a Bonga ewe delivers 12.2 ± 1.80 lambs in her lifetime which is lower than the 15.3 ± 4.3 lambs reported for Horro sheep (Edea et al., 2012). Bonga sheep are naturally managed in accelerated lambing rhythm which is shorter than the conventional year-round lambing schedule for sheep. Much of the above data backed by the findings of Edea (2008) point out that, most likely, Bonga sheep have a non-seasonal reproductive rhythm. In a recent report related to the community-based breeding program (CBBP) of the breed (Aynalem Haile, personal communication), lambing interval for 2,900 Bonga ewes under village conditions was estimated at 258.1 ± 1.14 days (nearly, 8.6 months). The same report provides further evidence to the relatively prolific trend in Bonga sheep with an average litter size of 1.54 ± 0.006 for a total of 10,814 observations under village conditions. Large variability characterizes the dataset with records varying between 1 and 4; village, year, season, age, and parity significantly affected the performances.

Globally, Bonga sheep breed seems to reproductively perform better than most other indigenous breeds in Ethiopia, particularly for litter size. Recently, our research conducted a selection signature analysis using genome-wide genotype data from the prolific Bonga sheep in Ethiopia and identified 41 candidate regions associated with fertility and reproduction traits. The analyses confirmed the presence of selection signatures in genomic regions that span or lay adjacent two genes, *GDF5* and *BMP15*, that are known to be associated with prolificacy (Dolebo et al., 2019). Nevertheless, the physiological mechanisms behind this high and variable performance remain unknown. To better understand the physiological mechanisms underlying expression of litter size in Bonga sheep, current study aimed at characterizing and comparing follicular dynamics, ovulatory response, and some ovarian endocrine attributes during the follicular and the luteal phases in synchronized Bonga sheep with different retrospective average records for litter size.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in Adiyo Kaka district of Kaffa zone. The district is located 509 km South West of Addis Ababa in Southern Nations, Nationalities and Peoples Regional State (SNNPRS). The study area is a wet, humid agro-ecology that lies at an altitude of 2,511 m above sea level, at 7°17′ N latitude and 36°24′ E longitude with temperature range 17.5–22.5°C. The area is characterized by large evergreen natural forest receiving rainfall almost year-round. The annual rainfall ranges from 1,700 to 2,000 mm with peak rainy season being mid-June to early October. The district has 35% highland, 55% midland, and 10% lowland agro-ecology. There are no ethical concerns to be reported and animals were handled in the presence of their owners by adhering to local animal practices and handling rules.

2.2 | Animals and experimental design

A total of 31 Bonga ewes aged between 4 and 5 years old and having a body condition score varying between 2.5 and 3.5 were selected from private farmers of Boka-Shuta CBBP cooperative, for the study. A subjective body condition scoring system was based on the scale of Russel, Doney, and Gunn (1969). The ewes were reared in small flock sizes of less than 10 ewes (Mamiru, Banerjee, & Haile, 2017) and were chosen based on existing litter size records for three consecutive lambing seasons with no history of reproductive disorders (extended lambing intervals, abortions, etc.) as per the database of the CBBP. Ewes were classified as high prolific (HP) (n = 20; average body condition score 3.1 ± 0.56) producing litter sizes ≥ 2 and low prolific (LP) (n = 11; average body condition score 3.0 ± 0.42) producing litter sizes equal to 1 during each of the three considered lambing seasons. All the ewes were confirmed to be non-pregnant using ultrasound-based pregnancy diagnosis.

Throughout the experiment, animals were assembled and kept in a community shed, grazed on natural grassland, had ad libitum access to clean water, and were exposed to natural daylight during the entire trial. Three sexually mature and experienced rams of the Bonga breed were used for estrus detection.

On November 5, 2017 estrous cycle was synchronized for the 31 selected ewes, using intravaginal progesterone sponges (Syncropart®; CEVA laboratories) inserted for 14 days. Determination of follicular dynamics was performed daily by transrectal ultrasonographic assessment of the number and size of all follicles ≥ 2 mm, from the day of sponge removal to the day following the onset of estrus (day of estrous being considered day 0 for uniformity of data presentation) or for 5 days following sponge withdrawal in those sheep that failed to exhibit estrus. Prior to echographic examination, all the ewes were subjected to 12-hr overnight fasting.

The presence and number of *corpora lutea* (CL) were also assessed by transrectal ultrasonography approximately 9 days following the onset of estrus.

2.3 | Assessment of preovulatory follicular development and the presence and number of corpora lutea

Ultrasonographic observations of the ovaries were performed by the same experienced operator using a 7.5-MHz transducer for

transrectal ultrasonography (Honda®, HS-2200V). After placing the sheep in the dorsal position as during laparoscopy, the probe was placed in the rectum with the transducer orientated perpendicularly to the abdomen wall using a hydrosoluble contact gel to enhance ultrasound transmission. Once the uterine horns were located, the probe was rotated laterally 90° clockwise and 180° counterclockwise to observe both ovaries and their structures after surpassing the urinary bladder (Gonzalez-Bulnes, Santiago-Moreno, Garcia-Lopez, Gomez-Brunet, & Lopez-Sebastian, 1994). Each ovary was scanned several times from different angles in order to image all follicles ≥ 2 mm. The largest diameter of each of these follicles was measured and its position was recorded on a paper-back diagram of each ovary. In order to ensure correct identification of all follicles at successive observations and follow their individual growth, both position and distance from the largest follicle and the ovarian medulla and hillus were recorded every day on the diagram of each ovary (Gonzalez-Bulnes, Souza, Campbell, & Baird, 2004).

Follicles recorded by ultrasonography from the left and right ovaries were classified as small [2–3 mm], medium [4–5 mm], and large [≥6 mm] and total follicles corresponding to all follicles larger than 2 mm. Follicles were further classified as atretic when being in a regressing phase (those that decreased in size between two successive ultrasound sessions) and new follicles (not previously detected; Cueto, Gibbons, Alberio, Taddeo, & Gonzalez-Bulnes, 2006).

Around day 9 following the onset of estrus, the left and right CLs were identified through their echogenic pattern and their numbers determined as described by Gonzalez-Bulnes, Santiago-Moreno, Gomez-Brunet, and Lopez Sebastián (2000).

2.4 | Estrus and mating

Sixteen hours following the removal of the intravaginal sponges, estrous behavior was detected at 8-hr intervals via direct observation of the ewes using three aproned teaser rams. Estrous detection continued for four consecutive days or until estrus was detected. Ewes standing to be mounted were considered to be in estrus and were mated with rams allocated at a mating ratio of 10 ewes to 1 ram. Each ewe was mated twice at 12 hr interval. Ewes not displaying estrus were also recorded.

2.5 | Assessment of estradiol and progesterone secretion

Blood sampling for estradiol determination took place every 8 hr, from 16 to 96 hr after sponge removal. This corresponded to the time period during which follicular dynamics and estrous behavior were being monitored. Blood was collected using vacutainer tubes infused with heparin (Vacutainer® Systems Europe, Becton Dickinson). For progesterone analysis, blood samples were collected at 48-hr intervals for 20 days following the removal of sponges. Samples were then immediately transported in a cooling box filled with ice to Bonga Research Center, Animal Health Laboratory, and centrifuged at 1,500 *g* for 15–20 min. Plasma, recovered from each centrifuged sample, was stored at −20°C for 3 weeks prior to undertaking progesterone and estradiol assays.

Plasma progesterone and estradiol concentrations were determined by enzyme-linked immunosorbent assay (ELISA) in duplicate using an ELISA assay kit (MyBioSource®) based on standard procedures following the manufacturer's instructions. The inter- and intra-assay variation coefficients were 8.6% and 11.2%, respectively, for progesterone and 6.2% and 10.3% for estradiol.

2.6 | Statistical analysis

For uniformity of variables, day 0 was equaled to be the day of onset of estrous. Day 0 in ewes that did not show estrus was assimilated to day 2 after the introduction of teaser rams on which more than 90% of the ewes displayed estrus. For changes in the frequency of follicular size, factorial ANOVA with two independent factors (time and prolificity) was used to test the difference between LP and HP ewes. The Students *t* test was used to compare differences in follicular numbers between the LP and HP ewes. One-way ANOVA was used to test differences in the number of CLs, atretic follicles, new follicles, and plasma concentration of estradiol and progesterone between the LP and HP ewes.

Mean number small, medium, large, total, atretic, and new follicles were expressed as mean ± *SEM*. Mean plasma estradiol concentrations and progesterone concentrations were expressed as mean ± *SD*. Statistical significance was set at $p < .05$ (Schwartz, 1993).

3 | RESULTS

3.1 | Estrous response

Following sponges' removal and the introduction of teaser rams, 27 of 31 ewes were detected to be in estrus in the following 3 days. Nine LP ewes (82%) were detected to be in estrus and 18 HP ewes (90%) were standing to estrus.

3.2 | Growth dynamics of preovulatory follicles

The changes in frequency of follicular size (mean number of small, medium, and large follicles) are presented in Figure 1.

The mean number of medium follicles tended to be higher in HP than in LP ewes ($p = .07$). In fact, in the HP group, the mean number of medium follicles increased from 0.89 ± 0.65 3 days prior to estrus to 4.89 ± 0.30 and 3.89 ± 0.30 at days −2 and −1 before estrus, respectively (Figure 1). Corresponding values in LP ewes remained lower (1.17 ± 0.70, 2.83 ± 0.37, and 1.83 ± 0.27 at days −3, −2, and −1 before estrus, respectively; Figure 1). Differences between LP and HP ewes were significant on days −2 and −1 preceding estrus

($p < .05$). However, for small and large follicles, no significant differences were observed between the two groups except on the day of estrus (day 0) when the mean number of large follicles was higher in HP compared to LP ewes (1.78 ± 0.19 and 1.0 ± 0.28 for HP and LP ewes, respectively; $p < .05$; Figure 1).

3.3 | Atretic and new follicles

Frequency distributions of small, medium, and large atretic and new follicles pooled together are reported. No differences were observed in the trend follicles were undergoing atresia. The mean number of new follicles was significantly higher in the ovaries of LP compared to those of their HP counterparts on the day of estrus and on the day prior to estrus ($p < .05$; Figure 2).

3.4 | Plasma Estradiol

For most of the sampling period, HP ewes had higher means of plasma estradiol but at some sampling points the difference failed to attain statistical significance because of high individual variations (Figure 3). Overall, the plasma estradiol concentrations were higher for HP than LP ewes (18.91 ± 0.41 vs. 14.51 ± 0.65 pg/ml; $p < .05$).

3.5 | Luteal function and plasma progesterone

The mean number of CL was significantly higher ($p < .05$) in HP (2.3 ± 0.15) than in LP (1.28 ± 0. 14) ewes. The mean plasma progesterone concentrations were also significantly higher ($p < .05$) in HP than in LP ewes (Figure 4) and these differences were more pronounced between days 10 and 15 following the removal of sponges. The largest difference was observed on day 12 when progesterone concentrations rose to an average value of 5.60 ± 0.71 ng/ml in HP ewes compared to 1.95 ± 1.63 ng/ml in LP ewes.

4 | DISCUSSION

The present study confirms that the LP and HP ewes of Bonga sheep exhibit different ovulation rates. The lambing data that were made available and consecutive to the following protocol is consistent with our hypothesis as LP ewes ($n = 7$) yielded an average litter size of 1.25 ± 0.433, while HP ewes ($n = 11$) produced 2.12 ± 0.499 lambs on average. Results of the current study suggest that differences in ovulation rate between the LP and HP ewes are caused by differences in the number of follicles growing to the large stage and the existence of co-dominance effects. Beyond the overall result confirming ovarian-related differences between LP and HP ewes, some features in the response of the two groups of sheep are very crucial.

It is important to highlight that the estrous response (87% of all experimental ewes) in this study was high. This was expected because the Bonga breed, like most tropical breeds of sheep, is not susceptible to changes in photoperiod and is commonly known to be non-seasonal. Although discrete periods of seasonality, resulting from other environmental and social cues, such as feed availability, ambient temperature, and disease incidences, can be reported for such breeds, it is anticipated that such effects would be negligible in the home region of Bonga sheep. Indeed, the area is characterized by mild temperatures and sufficient, well-distributed rainfall pattern over the year to maintain lush vegetation cover (Mamiru et al., 2017). With the exception of two individuals, all the sheep displayed estrus in a very synchronous way 2 days after the removal of sponges. The two exceptions, one LP and one HP had a delayed response on day

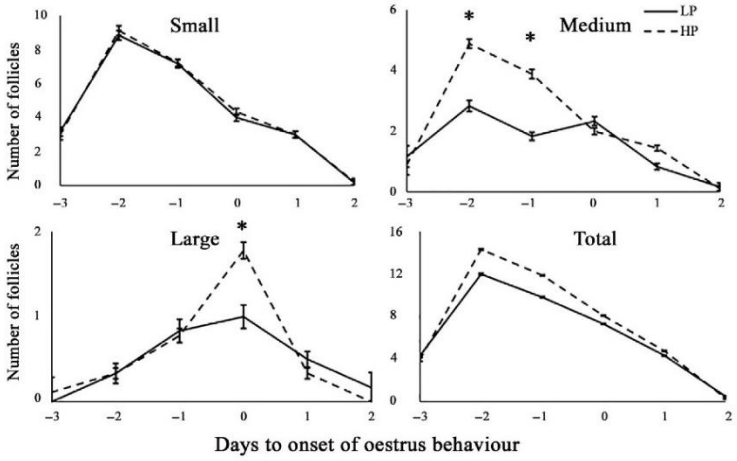

FIGURE 1 Mean number (±*SEM*) of small [2–3 mm], medium [4–5 mm], large ≥6 mm, and total follicles ≥2 mm during the follicular phase of Low (LP) and high prolific (HP) Bonga sheep. Statistically significant differences are indicated with * if $p < .05$

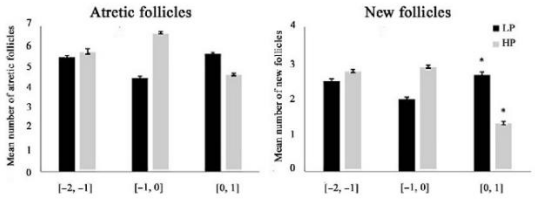

FIGURE 2 Mean number (±*SEM*) of atretic and new follicles for all follicular sizes during the follicular phase of Bonga ewes with single (LP) and multiple (HP) litter sizes. Day 0 corresponds to the day of onset of estrus. Statistically significant differences are indicated with * if $p < .05$

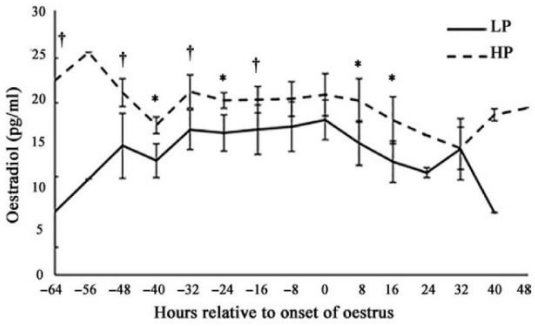

FIGURE 3 Plasma estradiol concentrations (mean ± *SD*) around estrus for Bonga ewes with single (LP) and multiple (HP) litter sizes. Statistically significant differences are indicated with * if $p < .05$ and † if $0.09 > p > .05$

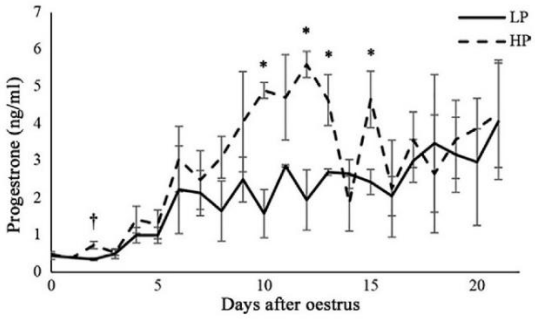

FIGURE 4 Plasma progesterone concentrations (mean ± *SD*) for Bonga ewes with single (LP) and multiple (HP) litter sizes every 2 days for 20 days after sponge removal. Statistically significant differences are indicated with * if $p < .05$ and † if $0.09 > p > .05$

3 after the removal of sponges. This observation is important as it discards, at least for HP Bonga ewes, the notion that increases in ovulation rate are related to an extended period of ovulatory follicle recruitment (Scaramuzzi & Radford, 1983). An extended period of follicle recruitment would allow a higher number of follicles growing to ovulatory stages as confirmed for various temperate sheep breeds (Bartlewski et al., 1999; Driancourt, 2001; Souza, Campbell, Webb, & Baird, 1997).

The number of small and medium follicles decreased during the follicular phase in both groups of ewes. This indicates that preovulatory follicles in both LP and HP ewes grew in a fast and continuous manner during the period of terminal growth, therefore exerting dominance over the remaining follicles. We also anticipate such a dominance effect of the preovulatory follicles to be higher in HP ewes due to the higher number of atretic follicles a day prior to estrus (although not significant) and the lower number of new follicles during the 24 hr following estrus. Such patterns of follicular growth are similar to those reported in other sheep breeds (Bartlewski et al., 1999; Ben Salem, Rekik, Gonzalez-Bulnes, Lassoued, & Kraïem, 2010; Evans, Duffy, Hynes, & Boland, 2000; Gonzalez-Bulnes et al., 2001; Lopez-Sebastian et al., 1997).

However, if we analyze the data obtained in the current study in chronological detail, it reveals some interesting trends. The mean number of CL in HP ewes is much higher than the average number of large follicles on the day of estrus. Furthermore, while the number of medium follicles declined slightly during the follicular phase in LP ewes, it rapidly declined in HP ewes over the 48 hr preceding estrus. Based on these two observations, it can be hypothesized that the medium follicles in HP ewes are able to be selected to ovulate, hence contributing to a higher ovulation rate. This agrees with the results of previous study that compared two strains of Tunisian Barbarine sheep (Lassoued, Rekik, Gonzalez-Bulnes, Ben Salem, & Tounsi, 2013) in which ewes of the prolific strain exhibited a codominance effect with several medium follicles ovulating. The existence of medium follicles contributing to the increase in ovulation rate supports the idea of codominance in sheep; i.e., the coexistence of two or more follicles, from the same or subsequent growth waves, reaching dominance and even ovulation (Bartlewski, Baby, & Giffin, 2011). Commonly, dominant follicles impair the development of smaller gonadotrophin-dependent follicles by suppressing FSH and inducing atresia (Campbell, Scaramuzzi, & Webb, 1995), but in sheep the dominance exerted by large follicles is weak (Gonzalez-Bulnes, Diaz-Delfa, et al., 2005a) and does not completely suppress ovulations from supplementary growing follicles of smaller sizes (Evans et al., 2000).

Throughout the follicular phase, estradiol secretion was consistently higher in HP ewes. This is probably due to the higher number of ovulatory follicles, but this does not preclude differences in functionality of the growing medium and large follicles. Such a hypothesis can only be verified with an appropriate experimental design to assess in vitro follicle competency, but it should be noted that estradiol is also a marker for follicular function (Souza, Campbell, & Baird, 1996) and, as a result, preovulatory (large and medium) follicles of the HP ewes have a higher responsiveness to gonadotrophin secretion, hence promoting their growth prior to ovulation. What should also be noted regarding estradiol pattern of secretion is the absence of the rise which should normally appear at estrus time in both HP and LP ewes. This can be argued by the negative effects of progestogens on the functionality of ovulatory follicles (Gonzalez-Bulnes, Veiga-Lopez, et al., 2005b). Indeed, they demonstrated that ovulatory follicles from sheep treated

with exogenous progestogens showed deficiencies in the secretion of estradiol during the preovulatory phase and no marked rise was detected at the time of estrus. This did not prevent estrus to be exhibited and ovulation to occur. However, in their study, subsequent corpora lutea secreted low progesterone.

None of the sheep had a short cycle on the basis of the echogenicity of the CL tissue and the screening of progesterone levels from day 2 to 14 following the withdrawal of sponges. The mean number of CL was significantly higher in HP than LP ewes. As the production of progesterone is done by the luteal cells, this could explain the significantly higher plasma progesterone concentrations in HP ewes during the mid-luteal phase (Mesen & Young, 2016). The overall reduction in plasma progesterone levels in HP ewes after day 14–16 could be explained by three ewes for which plasma progesterone levels declined to less than 0.4 ng/ml and remained undetectable until day 20. These three ewes most likely did not conceive to the synchronized estrus and underwent luteolysis.

In conclusion, this study provides evidence at the ovarian level of the phenotypic variability characterizing litter size in Bonga sheep found in Ethiopia. This is mainly reflected in the growth of larger preovulatory follicles and in the existence of co-dominance effects that could account for the difference in ovulation rates and litter sizes between LP and HP ewes.

ANIMAL WELFARE STATEMENT

The ethical polices of the journal have been adhered to, and that European Union (EU) standards on the protection of animal used for scientific purposes and/or feed legislation have been met.

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Paper III

Genome-wide scans identify known and novel regions associated with prolificacy and reproduction traits in a sub-Saharan African indigenous sheep (Ovis aries)

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Genome-wide scans identify known and novel regions associated with prolificacy and reproduction traits in a sub-Saharan African indigenous sheep (*Ovis aries*)

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Abstract
Maximizing the number of offspring born per female is a key functionality trait in commercial- and/or subsistence-oriented livestock enterprises. Although the number of offspring born is closely associated with female fertility and reproductive success, the genetic control of these traits remains poorly understood in sub-Saharan Africa livestock. Using selection signature analysis performed on Ovine HD BeadChip data from the prolific Bonga sheep in Ethiopia, 41 candidate regions under selection were identified. The analysis revealed one strong selection signature on a candidate region on chromosome X spanning *BMP15*, suggesting this to be the primary candidate prolificacy gene in the breed. The analysis also identified several candidate regions spanning genes not reported before in prolific sheep but underlying fertility and reproduction in other species. The genes associated with female reproduction traits included *SPOCK1* (age at first oestrus), *GPR173* (mediator of ovarian cyclicity), *HB-EGF* (signalling early pregnancy success) and *SMARCAL1* and *HMGN3a* (regulate gene expression during embryogenesis). The genes involved in male reproduction were *FOXJ1* (sperm function and successful fertilization) and *NME5* (spermatogenesis). We also observed genes such as *PKD2L2*, *MAGED1* and *KDM3B*, which have been associated with diverse fertility traits in both sexes of other species. The results confirm the complexity of the genetic mechanisms underlying reproduction while suggesting that prolificacy in the Bonga sheep, and possibly African indigenous sheep is partly under the control of *BMP15* while other genes that enhance male and female fertility are essential for reproductive fitness.

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Introduction

The evolution of novel traits is underpinned by genetic changes encoding new phenotypes. The genetic basis of traits that are controlled by few genes has been well established. For example, mutations in *MC1R* influences coat colour in animals ranging from mice (Hoekstra et al. 2006) to camels (Almathen et al. 2018). However, little remains known on the genetic control of complex traits, which have proven challenging to study using traditional approaches. Recent developments in next-generation sequencing and associated techniques (single-nucleotide polymorphism (SNP) genotyping arrays and bioinformatics pipelines) have provided a unique opportunity to examine genes, and gene networks, encoding complex phenotypes in domestic animals (Andersson and Georges 2004; Gouivea et al. 2014).

Diverse geographic adaptation and selection pressure have resulted in shared and population-specific phenotypes in many livestock species (Xu et al. 2015). Prolificacy is one such phenotype that has been observed in several

breeds of sheep in Europe, Africa, Middle East, and Asia and other mammalian species. Whether the trait evolved independently, within or across species and/or breeds of livestock in different geographic regions or, already existed in the genome of the wild ancestor at the time of domestication remains unknown. What is clear, however, is that the trait is under the control of a few genes with large effects (Davis 2004, 2005; Monestier et al. 2014; Abdoli et al. 2016). Several studies identified causative variants in three related oocyte-derived members of the transforming growth factor-beta (TGF-β) superfamily including bone morphogenic protein receptor 1B (*BMPR1B*), bone morphogenic protein 15 (*BMP15*) and growth differentiation factor 9 (*GDF9*) (Davis 2004, 2005; Abdoli et al. 2016) which have been shown to be essential for ovulation rate and follicular growth (Juengel and McNatty 2005; Knight and Glister 2006). The *BMPR1B* gene located on chromosome (Oar) 6 has been found in mostly Asian breeds. The sole mutation observed in this gene is present in the Small-tailed Han and Hu sheep in China, the Kendrapada and Garole sheep in India and the Javanese thin-tailed sheep in Indonesia but seems to be absent in European breeds (Davis et al. 2002, 2005; Jansson 2014; Abdoli et al. 2016). *BMP15* and *GDF9* located on OarX and 5, respectively, appear to be the main prolificacy genes in European and Middle East (specifically Iran) sheep breeds. In *BMP15* eight mutations, which differ slightly in type and effect, have been discovered in different sheep breeds and populations (see reviews by Davis 2004, 2005; Abdoli et al. 2016 and references therein). Four mutations affecting ovulation rate have been discovered to date in *GDF9* (see reviews by Davis 2004, 2005; Abdoli et al. 2016). Other genes that have also been reported in European sheep include *B4GALNT2* on Oar11 and *FecX2* on OarX (see Abdoli et al. 2016). New mutations are, however, continuously being discovered in these genes, the latest were reported in Tunisian Barbarine (Lassoued et al. 2017) and three Iranian breeds (Amini et al. 2018). These findings suggest the genetic control of prolificacy traits varies between breeds.

Sub-Saharan Africa (SSA) is home to several breeds of prolific indigenous sheep but with no known history, or information, on any form of either natural and/or artificial selection targeting the trait. In spite of the large body of knowledge generated in the last decade on prolificacy in domestic sheep, the genetic basis for the trait in SSA sheep remains poorly investigated. The various studies documenting variations in major genes and the inherent causative mutations associated with ovulation and litter size the species justify the investigation of genes of major effect in prolific SSA sheep. African indigenous sheep are known to share their genome ancestry with sheep from the Middle East and the Indian sub-continent (Muigai and Hanotte 2013; Mwacharo et al. 2017). The expectation therefore is that one of the three members of TGF-β superfamily of genes could be responsible for the trait in SSA sheep.

Bonga is a breed of indigenous sheep found in southwestern Ethiopia. It displays good maternal characteristics and is one of the naturally prolific breeds of sheep found in SSA. It shows an average litter size of 1.54 ± 0.006 (average range = 1.25 ± 0.433 to 2.12 ± 0.499) and above average reproductive efficiency under smallholder village production (Haile et al. Unpublished). Edea et al. (2012) reported an average litter size of 1.36 and average twinning rate of 36.3 ± 4.7% in the breed. Other naturally prolific breeds of sheep in SSA include Horro (litter size of 1.40 and twinning rate of 39.9%; Edea et al. 2012) and Doyogana breeds in Ethiopia, the West African Dwarf/Djallonke sheep found across southwest and Central Africa. Other prolific breeds of sheep in the continent outside SSA are the Barbarine and D'man sheep found in Tunisia and Morocco, respectively. The prolificacy in the Barbarine and D'man sheep has, however, been enhanced through artificial selection programmes.

In this study, we performed genome-wide scans of selection signatures using three approaches (F_{ST}, hapFLK, XP-EHH) and genotype data generated with the Illumina OvineHD SNP Chip array in Bonga sheep sampled from farmers' flocks, as a proxy for other prolific sheep found in SSA, to identify candidate genomic regions and genes associated with the trait. We identified a strong selection signature on OarX spanning *BMP15* and uniquely, a diverse range of genomic regions spanning several candidate genes, never reported before in prolific sheep, but known to be associated with fertility and reproduction in other species. Our results suggest that, prolificacy in SSA indigenous sheep is a function of the actions of *BMP15* and several genes that are associated with male and female fertility.

Results

The phenotypic dataset consisted of 98 litter size records of Bonga sheep, a non-seasonal breeder, that were collected between 2009 and 2018 from farmers flocks participating in a community-based breeding programme (CBBP). For this study, litter size was considered a prolificacy trait of the dam and one of the indicators of improved reproduction. It was defined as the number of lambs born alive per lambing. The most prolific ewes ($n = 74$) with twins ($n = 38$), triplets ($n = 35$) and quadruplets ($n = 1$) lambs born alive per lambing and non-prolific ones (one lamb born alive per lambing; $n = 24$), for at least three parities, were sampled from different farmers flocks. Genotyping was performed with the Illumina OvineHD BeadChip, which includes 606,006 genomic variants and 30,000 functional putative variants, at GeneSeek Inc (http://genomics.neogen.com/en/). The genotype data were assessed for quality with

PLINK 1.9 (www.cog-genomics.org/plink2). Variants with no assigned genomic positions, call rates lower than 95%, large Hardy–Weinberg equilibrium (HWE) deviations (P value $< 1 \times 10^{-6}$) and minor allele frequency (MAF) < 0.01, and samples with call rates $< 98\%$ were excluded from the final dataset. Following quality filtering, 457,087 variants and 84 individuals (33 ewes with twins, 30 with triplets, 1 with quadruplet, and 20 with single births) were retained for analysis.

To ensure that there were no biases attributed to stratification arising from fine-scale population genetic structure due to variations between and within farmers' flocks or any other unknown evolutionary attribute, principal component analysis (PCA) and NetView were performed using the retained genetic variants. No genetic stratification was detected (Figs. 1a, b) with the first principal component of the PCA explaining 80.92% of the total genetic variation. Irrespective of their prolificity (twinning, triplet, quadruplet), all the ewes clustered close together with only eight outliers (five ewes with triplets and two with singlets) being observed.

The retained dataset, following quality filtering, was used to investigate genome-wide signatures of selection using three cross-population selection tests; F_{ST} (Biswas and Akey 2006), XP-EHH (Sabeti et al. 2007) and hapFLK (Fariello et al. 2013). For the analysis, prolific ewes, defined as those with twin, triplet and quadruplet births, for at least three consecutive lambing seasons, were taken as one group and the non-prolific ones (ewes with single births) formed the contrasting group. The grouping was informed by the objective of detecting selection signatures that can be attributed, to a large extent, to differences in prolificacy. For this reason, we avoided using a different breed due to the high likelihood of detecting strong selection signatures arising from genetic differences between breeds which might have masked the ones attributing to prolificacy.

Based on the ovine RefSeq gene annotation, there were five and eight candidate regions revealed by XP-EHH and hapFLK, respectively, that overlapped no gene(s) (Table 1). For the candidate regions that overlapped with gene(s), two (one on Oar5 and the other on OarX; Table 1; Fig. 2a), were identified from the empirical genome-wide distribution of F_{ST} values. The region on Oar5 spanned 18 annotated genes and five novel protein coding transcripts, while the one on OarX, the most significant signature, spanned eight annotated genes and seven novel protein coding transcripts (Table 1). The XP-EHH detected 18 candidate regions, spanning 20 annotated genes and four protein coding transcripts, across 12 chromosomes (Table 1; Fig. 2b). The hapFLK revealed 21 candidate regions spanning 31 annotated genes and 13 protein coding transcripts, across 15 chromosomes (Table 1; Fig. 2c). The candidate region on OarX overlapped between F_{ST} and hapFLK tests. The *BMP15* gene, which has been implicated in prolificacy in several breeds of European and Middle East sheep (Davis 2004, 2005), occurred in this region within the most significant F_{ST} and hapFLK windows. The gene (*BMP15*), however, occurred 259,479-base pairs (bp) upstream of the region revealed by XP-EHH. A region on Oar13 that overlapped between hapFLK and XP-EHH spanned *DOK5* (Docking Protein 5) gene, an adapter intracellular protein that is involved in signal transduction and is expressed in lymphocytes and T cells in human and mice and may modulate various T cell functions (Favre et al. 2003). The *GDF9* gene occurred 4,456,484-bp downstream of the candidate region revealed by F_{ST} on Oar5. None of the three candidate regions identified by XP-EHH on Oar5 overlapped with *GDF9* or the F_{ST} region.

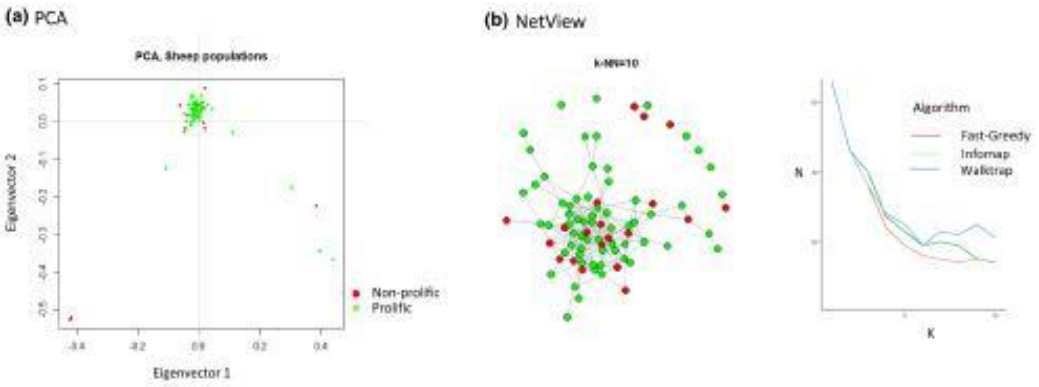

Fig. 1 Population cluster analysis of Bonga sheep as revealed using PCA and NetView

Table 1 Candidate regions and associated genes revealed by FST, hapFLK and XP-EHH selection signature analysis in the Bonga breed of sheep

Approach	Chromosome	Genomic region (bp)		Number of significant SNPs	Genes present
		From	To		
F_{ST}	5	46300001	47300000	168	**SPOCK1**, KLHL3, HNRNPA0, ENSOART00000016824, **MYOT**, **PKD2L2**, FAM13B, WNT8A, **NME5**, **BRD8**, KIF20A, CDC23, GFRA3, CDC25C, ENSOART00000017740, ENSOART00000014602, ENSOART00000017768, **KDM3B**, REEP2, EGR1, ENSOART00000017967, HSPA9, CTNNA1, HB-EGF, SLC4A9
	X	49700001	51100000		IQSEC2, KDM5C, **TSPYL2**, **GPR173**, **MAGED1**, GSPT2, **BMP15**, SHROOM4, ENSOART00000006108, ENSOART00000010008, ENSOART00000006154, ENSOART00000010178, ENSOART00000010185, ENSOART00000006171, ENSOART00000006186
hapFLK	1	128490718	128525811	10	APP
	2	119482589	119548236	7	–
		217377972	217398724	6	–
	3	12453207	12568175	17	ENSOART00000015088
		99126620	99326839	48	SLC9A4, IL18RAP, IL18R1, IL1RL1
	4	3548252	3582020	7	–
	8	41309054	41578812	41	MANEA
		63639731	63649616	4	ECT2L
	9	86323485	86432306	18	DECR1, NBN, OSGIN2
	11	21701005	21716773	8	GLOD4, ENSOART00000004387, GEMIN4, ENSOART00000012524
		54540051	54638872	29	**RNF157**, **FOXJ1**, EXOC7, ZACN, GALR2, SRP68, EVPL
	12	49122258	49208872	8	ENSOART00000003233, TMEM240, ENSOART00000003483, VWA1, TMEM88B, ANKRD65, MRPL20, CCNL2
	13	6331437	6352500	7	–
		81406516	81586998	36	DOK5
	14	12372110	12484709	20	ENSOART00000012708, FBXO31, ENSOART00000012902
	16	47181073	47266038	14	–
		60146782	60155318	2	–
	18	8519837	8539965	2	–
	20	35706919	35768326	13	CDKAL1
	24	17429910	17498671	12	–
	X	49938964	51146427	105	ENSOART00000010008, **MAGED1**, GSPT2, ENSOART00000006154, ENSOART00000010178, ENSOART00000010185, ENSOART00000006171, **BMP15**, ENSOART00000006186

Table 1 (continued)

Approach	Chromosome	Genomic region (bp)		Number of significant SNPs	Genes present
		From	To		
XP-EHH	1	32315627	32543171	17	–
		110286900	110307916	3	ENSOART00000009796, CD244
	2	217308731	217514986	14	MARCH4, **SMARCAL1**, ENSOART00000021
	3	27572804	27685620	4	TTC32, WDR35
		108990499	109273734	6	–
		165291738	165524251	13	AMDHD1, HAL, LTA4H, **ELK3**
	4	5379251	5380178	2	DDC
	5	18474732	18604264	8	SGTA, SLC39A3, DIRAS1
		51535099	51621099	4	ARHGAP26
		78895709	78945814	2	SSBP2
	6	74173229	74856423	8	–
	8	41273738	41354804	2	–
		74736476	74747842	2	MTHFD1L
	12	34094639	34154274	7	–
	13	81349010	81625427	6	DOK5
	21	18433779	18520811	5	ENSOART00000008010
	22	24231560	24329956	12	SORCS3
	X	50250146	50717975	16	**MAGED1**, GSPT2, ENSOART00000006154 (**BMP15** is downstream to the region)

Genes in bold are associated with either male or female reproduction and prolificacy

In total, we observed 73 annotated genes in 28 candidate regions that were identified to be under selection by F_{ST} (2 regions), XP-EHH (18 regions) and hapFLK (21 regions). Functional enrichment was performed with DAVID 6.3 (Huang et al. 2009a, b) resulting in four enriched clusters of genes (Supplementary Table S1). The top two clusters were associated with immune responses encompassing (i) Toll/interleukin-1 receptor homology (TIR) domain (enrichment score = 2.82) and (ii) immunoglobulin/immunoglobulin-like (IG) domain (enrichment score = 1.27). Protein–protein interactions (PPI) and gene ontology (GO) enrichments were investigated with STRING (Szklarczyk et al. 2019). The network proteins encoded by the 73 candidate genes had significantly more interactions among themselves than was expected for a random set of proteins of similar size drawn from the genome (33 edges identified; PPI enrichment P value = 0.00612; Fig. 3). STRING revealed three GO biological process terms that were the most enriched (Supplementary Table S2). The PFAM, InterPRO and SMART protein domains were all associated with Toll/interleukin-1 receptor (TIR) domain superfamily (Supplementary Table S3) while the SMART protein domains included immunoglobulin-like domains as one of the most enriched. The Reactome pathways were associated with interleukin-18 signalling (BTA-9012546; false discovery rate = 0.0492). Apart from *BMP15* and *GDF9*, that are known to be associated with prolificacy across a wide range of prolific sheep in Europe and the Middle East, literature mining identified several candidate genes associated with female and male fertility and reproduction functions (Table 1) in other species but which have not yet been reported in prolific sheep.

Discussion

In this study, we used the prolific Bonga sheep found in Ethiopia, as a proxy to other prolific indigenous African sheep which, apart from the thin-tailed West African Dwarf, are all fat-tailed and of the same genetic background (Muigai and Hanotte 2013) to investigate the genetic basis of prolificacy. We applied three methods, F_{ST}, hapFLK and XP-EHH, to exploit their strengths and increase the reliability of our findings by minimizing biases associated with each method (Simianer 2014). We were also unsure about

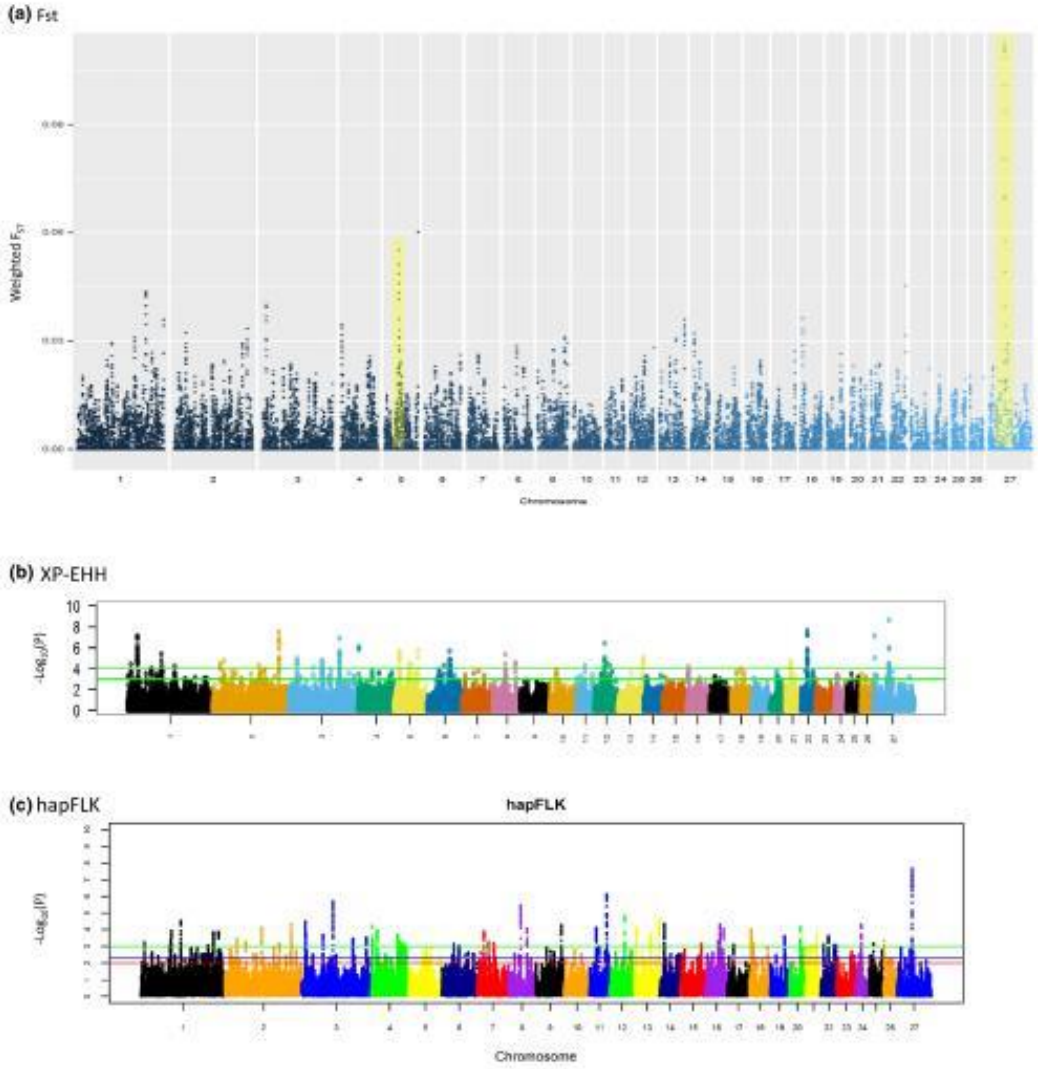

Fig. 2 Manhattan plots generated from the analysis of Ovine HD data generated in Bonga sheep

the evolutionary selection time span of prolificacy and the best method to detect candidate selection signatures associated with the trait. The F_{ST}, which is directly related to variance(s) in allele frequency, is the most commonly used method to detect selection signatures. The hapFLK detects selection signatures based on differences in haplotype frequencies while accounting for hierarchical population structure. XP-EHH detects long-range haplotypes or recent positive selection, where the selected loci are close to fixation in one population but remain polymorphic in another based on the relationship between an allele and its surrounding linkage disequilibrium (LD). Approaches such as F_{ST} and hapFLK detect better long-term selection because they are dependent on the accumulation of mutations around the causative variant. Their resolving power, however, declines if the selection advantage is small, as it takes longer for the frequency of the favoured allele to increase to the point of detection. LD-based methods, such as XP-EHH, retain

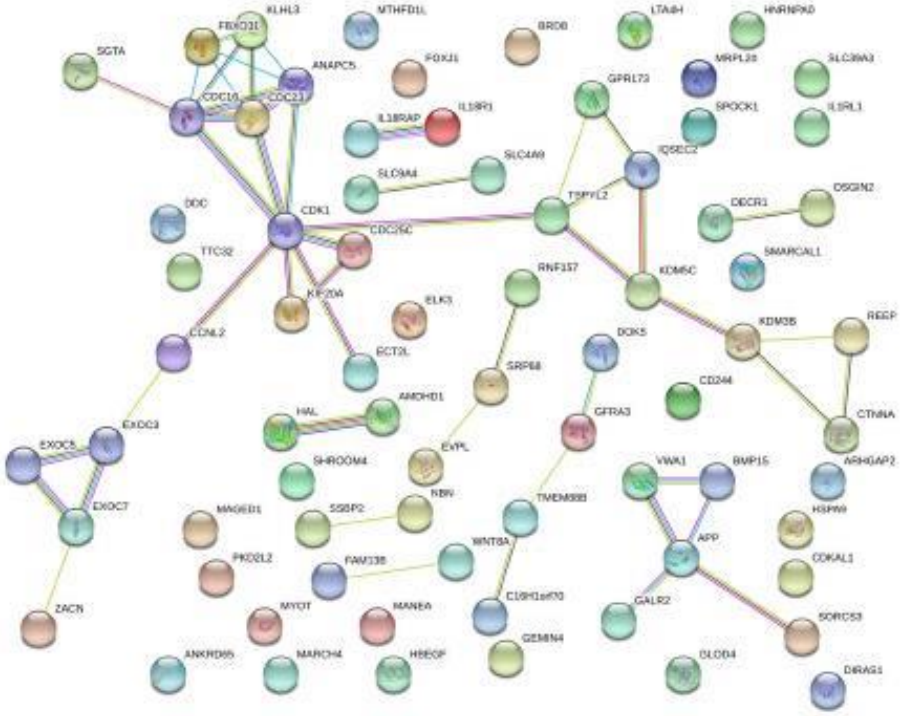

Fig. 3 Protein–protein interactions (PPI) network for the 73 candidate genes as revealed by STRING

superior detection power when a new mutation arises in a population due to an adaptive advantage or an existing variant being exposed to a new environment that provides favourable selection pressure, resulting in an increase in its frequency but without fixation (Fagny et al. 2014).

Functional enrichment analysis using the highest classification stringency in DAVID 6.8 revealed four enriched clusters with genes linked to immune functions being over-represented, a result which was replicated by STRING. The importance of the immune system in reproduction in sheep has recently been reported (Pokharel et al. 2019). Following arguments advanced in other studies that found an over-representation of immune-related genes in the genomes of tropically adapted livestock (Xu et al. 2015; Bahbahani et al. 2017; Mwacharo et al. 2017), it can also be argued that selection has enhanced adaptive immune response to tropical infections in the genomes of Bonga sheep. This is possible, but unlikely to explain the result of our study given its analytical design which contrasted prolific and non-prolific ewes of the same breed from the same ecological environment and therefore exposed to similar pathogens, parasites and infections. An alternative explanation or hypothesis that we favour associates the immune function with enhancement of the process and success of fertilization in the female reproductive tract. As in other eutherians, in domestic sheep, fertilization also occurs internally and ejaculated spermatozoa are normally retained in functional pre-ovulatory sperm reservoirs in female genital tracts until ovulation. Rather than being eliminated, the immunologically foreign sperm is tolerated by the female immune defence system. It has been observed that semen can signal genomic shifts that modulate expression of genes linked to immune function in poultry (Das et al. 2009; Long et al. 2003; Huang et al. 2016) and mammals (Almiñana et al. 2014; López-úbeda et al. 2015), resulting in a state of immune tolerance during the lengthy storage of spermatozoa in oviductal sperm reservoirs (Holt and Fazeli 2016). A large subset of differentially expressed genes that suppress local immune defence in oviductal sperm reservoirs following mating and mating-induced changes in the expression of immune activating genes in utero-tubal junction has also been reported in chicken and pigs, respectively (Atikuzzaman et al. 2017). The upregulation of immune defence genes in the oviduct following mating was also observed in mice (Fazeli et al. 2004). The

interval between sperm deposition and the change in gene expression seems to cover the time period when spermatozoa are in the sperm reservoir. The activation of immune response genes therefore serves to cleanse the genital tract of redundant spermatozoa, foreign proteins and/or pathogens, for the descending ova or embryos. It is therefore a possibility that high prolificacy has made the oviduct of prolific individuals less responsive to antigenic seminal fluid. This creates an appropriate immune-balanced physiological environment tailored for sperm survival and fertilization.

The F_{ST} and hapFLK identified one overlapping candidate region on OarX. As was expected based on our sampling and analytical design, the most significant window in this region spanned *BMP15* that plays a role in various functions implicated in prolificacy (Persani et al. 2014; Monestier et al. 2014). The candidate region revealed by XP-EHH on OarX was 259,479-bp downstream of *BMP15*. The F_{ST} test also revealed a region on Oar5 which was 4.4 Mb downstream of *GDF9*, an important paralog of *BMP15*. As the extent of LD declines rapidly from 0 to 300 kb in the ovine genome (Kijas et al. 2014; Al-Mamun et al. 2015), LD between the SNPs found on the XP-EHH candidate region on OarX and *BMP15* is thus expected. This result suggests that BMP15 may be the primary candidate gene responsible for prolificacy in Bonga sheep. Experimental disruption of *BMP15* in mice result in mild defects in female fertility (Su et al. 2008) whereas natural missense mutations result in variable phenotypes in ewes, ranging from hyperprolificacy to complete sterility, depending on a fine gene dosage mechanism involving *GDF9* (Belli and Shimasaki 2018). This is the first study, to the best of our knowledge, to identify a candidate genomic region and gene associated with prolificacy in an indigenous SSA sheep breed. It is therefore possible that *BMP15* may also encode the trait in other prolific SSA sheep. It would be of interest to investigate whether the causative variant(s) are the same or novel across prolific SSA sheep breeds *vis a vis* the ones found in Europe and the Middle East. This will shed light on the evolution of the genetic basis of the trait and is of relevance since at least 8 mutations have been reported, so far, in *BMP15* while a new variant was recently found in the Barbarine (Lassoued et al. 2017) and three Iranian (Amini et al. 2018) breeds.

The study design also revealed genes, not reported previously in prolific sheep, but have been associated with female and male reproduction traits in other species. The ones reported in female animals included *SPOCK1*, *PKD2L2*, *HB-EGF*, *GPR173*, *MAGED1*, *SMARCAL1*, *HMGN3a*, *ELK3* and *KDM3B*. *SPOCK1* was identified as a novel candidate gene for age at start of menstruation, which marks the beginning of a females reproductive life (Dvornyk and Waqar-ul-Haq 2012), in beef cattle (Fortes et al. 2010) and humans (Liu et al. 2009). With its specific expression in postnatal day-1 to postnatal day-14 ovaries, and low, albeit significantly in adult ovaries in mice, *PKD2L2* possibly functions in early or late follicular growth or at multiple stages of follicular development to regulate the assembly, preservation and maturation of ovarian follicles (Gallardo et al. 2007). In vitro studies showed possible interactions of *HB-EGF* with blastocyst epidermal growth factor receptor (*EGF-R*) very early in the process of implantation in species with different hormonal requirements (Das et al. 1994; Martin et al. 1998; Mishra and Seshagiri, 2000; Seshagiri et al. 2002). It is possible therefore that *HB-EGF* could be a critical signalling protein for early pregnancy success (Das et al. 1994; Paria et al. 2001; Xie et al. 2007; Jessmon et al. 2009). *GPR173* is a receptor for *PNX*, an important mediator of ovarian cyclicity and through its actions at multiple levels of the hypothalamo–pituitary–gonadal axis, it is involved in maintaining various reproductive functions in rats (Stein et al. 2016; Treen et al. 2016; Bauman et al. 2017) and possibly in prolific sheep. The expression of *MAGED1* has been detected in mice embryos as early as E3 stage (Kendal et al. 2002). Similarly, the expression of *SMARCAL1* and *HMGN3a* has been detected in bovine oocytes and in early embryos (Uzun et al. 2009), whereas *ELK3* has been isolated from 16-day mouse embryos and one of its transcripts was expressed predominantly in 8- to 14-day embryos (Nozaki et al. 2009). These expression patterns highlight the importance of these genes in regulating gene expression, during embryonic development. The *KDM3B* gene is highly expressed in female reproductive organs including the ovary, oviduct and uterus. Knockout of *Kdm3b* in female mice resulted in irregular oestrus cycles and decreased ovulation capability, fertilization rate, uterine decidual response and circulating levels of 17β-estradiol (Liu et al. 2015a). Thus *KDM3B* could play a crucial role in regulating oestrus and menstrual reproduction cycles and in preparing the uterus for successful implantation of multiple ova.

The candidate regions identified in this study also spanned several genes implicated in male fertility and reproduction in other species, but not reported in prolific sheep. They included *FOXJ1*, *NME5*, *PKD2L2*, *MAGED1* and *KDM3B*. As discussed, the latter three genes have been implicated in female reproduction. *FOXJ1* is a key transcription factor for the formation of motile cilia in human (Lyons et al. 2006), mice and xenopus (Weidemann et al. 2016). Immotile-cilia syndrome has been associated with male and female infertility in humans as motile cilia are critical in propelling ova along the fallopian tube while motility in sperm flagellum is also critical for sperm function and successful fertilization (Afzelius and Eliasson 1983). In mice and xenopus, *CFAP157* was identified as a novel target protein for *FOXJ1* and is only essential during spermatogenesis but is expressed in motile ciliated tissues. The prominent expression of *PKD2L2* and its encoded protein Polycystin-L2 in adult mouse testis, spermatocytes and spermatids

(Guo et al. 2000) suggests a role in spermatogenesis (Chen et al. 2008) and testicular maturation (Kierszenbaum 2004). Polycystin-L2 also modulates intracellular calcium concentration during spermatogenesis. Calcium ions are critical in regulating sperm cell functions including capacitation, progressive motility, hyperactivated motility and acrosome reaction, which are important during fertilization (Bedford 1998; Darszon et al. 1999; Zhang and Gopalakrishman 2005). *NME5* also exhibits a strict testis-specific expression in spermatogonia and early spermatocytes in several vertebrates (Muneir et al. 1998; Hwang et al. 2003; Desvignes et al. 2009). The protein encoded by *KDM3B* is also highly expressed in multiple cell types in mouse testes, such as Leydig and sertoli cells, spermatogonia and spermatocytes, at different stages of differentiation and has also been observed in epithelial cells of the caput epididymis, prostrate and seminal vesicle (Liu et al. 2015b). Knockout of *Kdm3b* in male mice resulted in reduction in the number of pups produced by breeding pairs due to a decrease in the number of litters, fewer number of mature sperms in cauda epididymis displaying significantly reduced sperm motility and a significant reduction in circulating levels of 17beta-estradiol, a modulator of sperm maturation and male sexual behaviour. Like *Kdm3b* knockout males, *MAGED1*-deficient male mice also displayed severely impaired male coital behaviour resulting in male infertility which is attributed to deficient production of mature oxytocin in hypothalamus indicating that *MAGED1* is required for oxytocin processing and stability (Dombret at al. 2012). The occurrence of these genes in the candidate regions suggests that they are essential in the maintenance of the process of spermatogenesis and normal male sexual behaviour to ensure successful fertilization of a large number of ova generated in prolific ewes.

Conclusions

The overall objective of this study was to identify genes associated with prolificacy in a prolific SSA fat-tailed breed of sheep. Unexpectedly, we also identified several known and novel candidate genes implicated in male and female fertility and reproduction in other species, suggesting that such genes could be hotspots of selection in indigenous SSA prolific breeds of sheep. The findings suggest that enhanced reproduction in prolific ewes entails not only prolificacy genes but also epistatic effects with genes associated with other reproduction traits. Although we identify *BMP15* as the main candidate gene for prolificacy in Bonga sheep, the exact causative variants need to be determined to further confirm whether they are novel or are part of what has been reported in prolific breeds of sheep from Europe and the Middle East. It is important to note that the sample size used here, 84 individuals, is rather low. This may have underpowered our analysis; thus, our findings should be interpreted with caution and need validation using a larger subset of animals and populations. Our findings are of significance given that reproductive traits have low to medium heritability and thus do not exhibit a noticeable response to phenotypic selection in traditional breeding methods based on phenotypic data only. The incorporation of the genetic information, such as revealed here, in such breeding programmes (e.g. CBBPs) via either genomic selection (GS), marker-assisted selection (MAS), genome-wide association studies (GWAS) or genomic best linear unbiased predictions of breeding values, could enhance response to selection towards the genetic improvement of reproductive performance.

Materials and methods

Samples, genotyping and quality control

A total of 95 ewes belonging to the Bonga breed of sheep were sampled from four locations (Shuta $n = 33$, Boqa $n = 45$, Buta $n = 13$, Medudha $n = 4$) in Southwestern Ethiopia. All the 95 animals had at least three lambing parities and came from farmers flocks that are participating in a community-based breeding programme (CBBP) where performance recording is undertaken. From the records of the 95 animals, 31 gave birth to single lambs, 33 to twins, 30 to triplets and one to a quadruplet. Whole blood was collected from each animal via jugular venipuncture with EDTA as the anticoagulant and later transferred to Whatman™ FTA™ Classic Cards (GE Healthcare) for storage. Genotyping was done using FTA™ preserved blood samples with the Ovine Infinium® HD SNP BeadChip (http://genomics.neogen.com/en/illumina-ovine-hd-beadchip) at GeneSeek Inc. (Lincoln NE, USA). The BeadChip includes 606,006 genomic variants designed by the International Sheep Genomics Consortium (ISGC), nearly all the contents from the original OvineSNP50 array and 30,000 putative functional variants. The raw genotypes were first analysed with GenomeStudio (Illumina; GenCall score for raw genotypes > 0.15) which was used to extract the genotypes in standard format for PLINK 1.09 (http://www.cog-genomics.org/plink/1.9/). Chromosomal coordinates for each SNP were obtained from the Ovine Oar 3.1 genome assembly (https://www.ensembl.org/Ovis_aries/Info/Index). The raw dataset was filtered to remove animals with more than 10% missing genotypes, SNPs with no known positions in the genome, SNPs with a call rate lower than 95%, a minor allele frequency lower than 1%, and large HWE deviations ($P < 0.000001$). All the SNPs mapping to the X chromosome were retained in the final dataset because genes associated with prolificacy have also

been observed on this chromosome. Hence, 84 individuals and 457,086 SNPs were available for analysis following quality filtering. This dataset was first used to assess genetic relationship and structure by performing the principal component analysis (PCA) with ADEGENET (http://adegenet.r-forge.r-project.org/) executed in R (https://www.R-project.org). To further assess possible fine-scale population genetic structure, NetView (Neuditschko et al. 2012) was also used to analyse the genotype data, testing up to ten near-neighbour genetic clusters.

Identification of candidate genomic regions under selection

Three selection detection tests, F_{ST}, hapFLK and XP-EHH, were implemented. Prior to performing selection signature mapping, the genotyped individuals were classified, a priori, into two groups, prolific and non-prolific ewes. The prolific group included ewes with twins, triplets and quadruplet litter sizes. The non-prolific group included ewes with single litter sizes.

Smoothed F_{ST} test statistic

The F_{ST} test indicates genetic differentiation among groups of individuals/populations/breeds arising from different evolutionary pressures acting in a segment of the genome. To identify loci under selection, we calculated the allele frequencies for each of the 457,086 retained SNPs for the two contrasting groups of prolific and non-prolific ewes. The allele frequencies were used to calculate F_{ST} values for each locus as a measure of group differentiation following Porto-Neto et al. (2013a, b). For each SNP, F_{ST} was calculated as the squared deviation of the average frequency in a group from the average frequency across the groups divided by the allele frequency variance (p*q). To identify regions under selection, the non-prolific group was compared against the prolific one and the pairwise group values were averaged to obtain a single F_{ST} value per SNP for each group. To facilitate the identification of genomic regions containing more extreme F_{ST} values, the individual SNP values of F_{ST} were grouped within genomic windows, using a kernel regression smoothing algorithm (Gasser et al. 1991) implemented with LOKERN in R (Hermann 2016). This method uses local averaging of the observations (F_{ST} values) when estimating the regression function. By testing window sizes of two, five and ten SNPs, we chose a window of five SNPs as it gave sufficient smoothing and showed the best signals. Higher scores of smoothed F_{ST} for individual loci or genomic regions indicate stronger signal of genetic differentiation/selection. Smoothed F_{ST} values greater than the average plus/minus three standard deviations (mean $F_{ST} \pm 3$ SD) were taken to be under selection.

hapFLK test statistic

As a complementary approach to mapping selection sweeps, we used hapFLK 1.3 (https://forge-dga.jouy.inra.fr/projects/hapflk), which implements the FLK (Bonhomme et al. 2010) and hapFLK (Fariello et al. 2013) algorithms. The FLK tests the neutrality of polymorphic markers by contrasting their allele frequencies in a set of populations against what is expected under neutrality. The hapFLK extends the FLK test to account for differences in haplotype frequencies between populations. This method has been shown to be robust with respect to bottlenecks and migration events (Fariello et al. 2013). To perform hapFLK analysis, Reynolds' genetic distances between the prolific and non-prolific ewes were calculated and converted to a kinship matrix with an R script (available at https://forge-dga.jouy.inra.fr/projects/hapflk/documents). Subsequently, by assuming ten haplotype clusters in the linkage disequilibrium (LD) model (− K 10; number of haplotype clusters determined by running a fastPHASE cross-validation analysis), the hapFLK statistics were computed and averaged across 20 expectation–maximization runs to fit the LD model (− nfit = 20). The standardization of the statistics using the corresponding python script provided with the software allowed the estimation of the associated P values from a standard normal distribution. To correct for multiple testing, we considered the threshold of the nominal P value as < 0.001 to identify the significant haplotypes following previous studies using hapFLK analysis on the Sheep HapMap dataset (Fariello et al. 2014; Kijas 2014).

XP-EHH test statistic

We also used the SelScan package (Szpiech and Hernandez 2014) to perform an additional analysis based on the cross-population extended haplotype homozygosity (XP-EHH) test (Sabeti et al. 2007). This statistic compares the EHH profiles for bi-allelic SNPs between two populations rather than two alleles in a single population. It is defined as the log of the ratio of the integrals of the EHH profiles between the two populations. It is calculated as:

$$\text{Unstandardized XP-EHH} = \ln\left(\text{iHH}_{\text{A}} / \text{iHH}_{\text{B}}\right)$$

where iHH_{A} and iHH_{B} are the integrated EHH of a given core SNP in population A and B, respectively. The comparison between populations normalizes the effects of large-scale variation in recombination rates on haplotype diversity

and has a high statistical power to detect sweeps that are close to fixation (Sabeti et al. 2007). We used the software developed by Pickrell et al. (2009) to estimate unstandardized XP-EHH statistics using all the SNPs that were retained following quality control. The unstandardized XP-EHH statistics were standardized using their means and variances in the comparison. Because previous studies found that the standardized XP-EHH statistics follow the standard normal distribution (Sabeti et al. 2007; Ma et al. 2014; Zhao et al. 2016), the *P* values for SNPs were estimated using the standard normal distribution function. Positive and negative XP-EHH estimates indicated positive recent selection in prolific and non-prolific ewes, respectively. For consistency with the threshold used for hapFLK, we considered as significant those positions showing *P* values < 0.001.

Functional enrichment of the candidate regions under selection

For the three selection mapping approaches, positions that showed evidence of selection (mean $F_{ST} \pm 3$ SD; or a *P* value < 0.001 for hapFLK and XP-EHH) were considered to be the result of selection sweeps. The genes that were either partially or fully covered by these regions were identified based on the ovine 3.1 reference genome assembly using Ensembl Comparative Genomics Resources Database Release 94 (https://www.ensembl.org/index.html). Functional enrichment analysis was performed with the functional enrichment clustering tool of DAVID Bioinformatics Resources 6.8 (Huang et al. 2009a, b). Each gene was analysed and enrichment analysis was performed using *Ovis aries* as the target species and the *Bos taurus* genome supplied with DAVID 6.8 as the background species. Corrections for multiple testing were performed by applying the Benjamini and Hochberg (1995) approach. For functional enrichment clustering, an enrichment score of 1.3 was taken as the threshold following the authors of DAVID 6.8. A search of the literature was also performed to identify phenotypes that are known to be affected by variation in the genes found in the candidate regions in other species.

Functional protein–protein interaction (PPI) networks and gene ontology (GO) terms encoded by the candidate genes were also investigated using STRING Genomics 11.0 (Szklarczyk et al. 2019) with the *Bos taurus* as the background species. STRING provides (i) known PPI from curated databases or experiments and (ii) PPI predicted on the basis of gene neighbourhoods, fusions and co-occurrences, text mining in literature, co-expression or protein homology. A global PPI network which retained interactions with a high level of confidence (PPI enrichment score > 0.4) was constructed.

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Author contributions MR, AH, BR and JMM contributed to the study conception and design. On-farm phenotypic recording and tissue sample collection were done by ATD and AM. Data analysis was done by NK, DW and JMM. Genotyping reagents were provided by MFR. The study was supervised by JMM who also wrote the manuscript. All authors read and approved the final manuscript.

Data availability The datasets analysed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical standards

Conflict of interest The authors declare no conflict of interest.

Research involving human and animal participants The animals used in this study are owned by farmers who are participating in a community-based indigenous sheep breeding programme. The farmers were aware of the objectives of the study and the animals were sampled with their permission in the presence of a qualified veterinarian following the guidelines for the care and use of animals of the Ministry of Livestock and Fisheries Resources, Peoples Democratic Republic of Ethiopia. No animal was injured during the sampling process. All the procedures described here were approved by the Institute Ethics Committee of the International Centre for Agricultural Research in the Dry Areas (ICARDA).

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Supplementary Table 2

Gene Ontology Biological Process Terms

# term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (IDs)	matching proteins in your network (labels)
GO:0006547	histidine metabolic process	2	3	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0015942	formate metabolic process	2	2	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0019556	histidine catabolic process to glutamate and formamide	2	2	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0019557	histidine catabolic process to glutamate and formate	2	2	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0043606	formamide metabolic process	2	2	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0043648	dicarboxylic acid metabolic process	3	33	0.0393	ENSBTAP00000021647,ENSBTAP00000021649,ENSBTAP00000052260	AMDHD1,HAL,MTHFD1L

GO:0052803	imidazole-containing compound metabolic process	2	4	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0052805	imidazole-containing compound catabolic process	2	4	0.0393	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL
GO:0043603	cellular amide metabolic process	6	316	0.0473	ENSBTAP00000006406,ENSBTAP00000021647,ENSBTAP00000021649,ENSBTAP00000021833,ENSBTAP00000028372,ENSBTAP00000052260	AMDHD1,HAL,LTA4H,MRPL20,MTHFD1L,VWA1

Supplementary Table S3

PFAM Protein Domain

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (IDs)	matching proteins in your network (labels)
PF01582	TIR domain	3	18	0.0057	ENSBTAP00000001371,ENSBTAP00000024715,ENSBTAP00000045070	IL18R1,IL18RAP,IL1RL1
PF13432	Tetratricopeptide repeat	3	37	0.0204	ENSBTAP00000011540,ENSBTAP00000020081,ENSBTAP00000020704	CDC23,SGTA,TTC32

Inter ProProtein Domains

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (IDs)	matching proteins in your network (labels)
IPR015621	Interleukin-1 receptor family	3	10	0.0022	ENSBTAP00000001371, ENSBTAP00000024715, ENSBTAP00000045070	IL18R1,IL18RAP,IL1RL1
IPR001057	Toll/interleukin-1 receptor homology (TIR) domain	3	20	0.0067	ENSBTAP00000001371, ENSBTAP00000024715, ENSBTAP00000045070	IL18R1,IL18RAP,IL1RL1

IPR035897	Toll/interleukin-1 receptor homology (TIR) domain superfamily	3	22	0.0067	ENSBTAP00000001371, ENSBTAP00000024715, ENSBTAP00000045070	IL18R1,IL18RAP,IL1RL1
IPR004074	Interleukin-1 receptor type I/II	2	6	0.0163	ENSBTAP00000001371, ENSBTAP00000024715	IL18R1,IL1RL1

Smart Protein Domains

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (IDs)
SM00255	Toll - interleukin 1 - resistance	3	15	0.0016	ENSBTAP00000001371,ENSBTAP00000024715,ENSBTAP00000045070
SM00410	Immunoglobulin like	4	103	0.0129	ENSBTAP00000001371,ENSBTAP00000003843,ENSBTAP00000024715,ENSBTAP00000045070

Reactome Pathways

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network (IDs)	matching proteins in your network (labels)
BTA-9012546	Interleukin-18 signaling	2	5	0.0465	ENSBTAP00000001371,ENSBTAP00000045070	IL18R1,IL18RAP
BTA-70921	Histidine catabolism	2	8	0.0495	ENSBTAP00000021647,ENSBTAP00000021649	AMDHD1,HAL

11. BIOGRAPHICAL SKETCH

Asrat Tera Dolebo was born in June 1972 at Woybo kebele of Bolosso Sorrie District, Wolaita Zone. He attended his primary education at Woybo Elementary School; and secondary education at Dubbo and Senior Secondary at Areka and Soddo Comprehensive High Schools. He then joined the Addis Ababa University, Awassa College of Agriculture in 1991 and graduated in 1993 with diploma in the field of Animal Science and Technology. Soon after his graduation, he was employed by the Ministry of Agriculture and served as Animal Production expert at Kamba Woreda Agricultural Office, Gamo Zone of SNNPR. He then, admitted to advanced standing program of the former Awassa College of Agriculture in 1997 and graduated with Bachelor of Science degree in the field of Animal Production and Rangeland Management in July 1999. After completion of his study, he was employed by the Wolaita Zone Department of Agriculture and served at different positions as Animal Husbandry expert at Soddo Zuriya Woreda Office of Agriculture, Wolaita Zone Agricultural Development Department and deputy administrator and head of Agricultural and Rural Development at Bolosso sorie woreda for five years. In February 2005, he joined Hawassa University to pursue his M.Sc. study in Animal and Range Sciences and graduated with Master of Sciences degree specializing in Animal Production in July 2007. He was then assigned as Academic vice Dean of Wolaita Soddo Agricultural Technical Vocational Education and Training College where he served for about three years. In the year 2011, he was assigned by the Regional Government as a Livestock Research Director at South Agricultural Research Institute and served there for about five years. Later he was assigned by the Government as a Head of Livestock and Fisheries Resources in SNNPR and worked for about two years. In November 2014, he joined the School of Graduate Studies of Hawassa University to pursue hisPhD study specializing in Animal Breeding and Genetics at the School of Animal and Range Sciences.