# Reproductive and Lactation Performance of Dairy Cattle in the Oromia Central Highlands of Ethiopia

## with Special Emphasis on Pregnancy Period

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# Abstract

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This thesis presents studies of the reproductive and lactation performance of dairy cattle in the Oromia Central Highlands of Ethiopia, with particular emphasis on diagnosing and monitoring pregnancy. In an introductory study, early abortions were induced in four heifers with cloprostenol and monitored using ultrasonography. Prior to cloprostenol injection, plasma concentrations of bovine pregnancy-associated glycoprotein 1 (bPAG1), prostaglandin $F_{2\alpha}$ (PG metabolite), and progesterone (P4) varied from 8.4–40 ng/mL, 158–275 pmol/L, and 20.7–46.9 nmol/L, respectively. After foetal death and subsequent expulsion, the bPAG1 level began to decrease, though the decrease was small and gradual. The PG metabolite level started to display short lasting peaks within three hours, while the P4 level dropped sharply. A cross-sectional study was followed by two years of longitudinal observation to examine the reproductive and lactation performance of smallholder dairy cows in Selalle, central Ethiopia: these studies involved 300 and 24 randomly selected smallholder dairy farms, respectively. The geometric means of the calving-to-conception interval (CCI) and duration after last calving as determined in the cross-sectional study were 187 and 201 days in pregnant and non-pregnant cows, respectively. While, the means of calving interval (CI), CCI and first observed oestrus after calving as determined by the longitudinal observations were 516, 253, and 141 days, respectively. Production system, district (location), and suckling status influenced these intervals considerably. Based on milk P4 profiles, the mean first onset of luteal activity (OLA) after calving was 52 days and was significantly influenced by suckling and parity number. The prevalence of pre-weaning calf mortality was quite high, up to 17.4%. The mean lactation length was 54.4 weeks, while the mean milk yield for the first 43 weeks of lactation was 11.7 L/day for crossbred cows. Suckling status, season of calving, and parity number significantly influenced the daily milk yield. A further follow-up study described the serum profiles of pregnancy-associated glycoprotein (PAG), oestrone sulphate (E1-S), and P4 during gestation in both Borana and crossbred cattle. Accordingly, the PAG concentrations at the $4^{th}$ week post conception were 1.5–5.5 ng/mL and 2.1–4.7 ng/mL in Borana and crossbred cattle, respectively, and increased progressively, peaking approximately at the time of calving. The serum E1-S concentrations at the $17^{th}$ week of pregnancy were 0.3–2.6 and 0.9–5.7 ng/mL in Borana and crossbred cattle, respectively, and increased progressively until calving. Breed, parity status, dam body weight, foetal sex, and foetal birth weight (only for PAG) significantly influenced PAG and E1-S concentrations. The P4 concentrations at the $4^{th}$ week of pregnancy were 3.2–5.1 and 1.7–8.9 ng/mL in Borana and crossbred cattle, respectively, and remained elevated throughout gestation. In conclusion, the smallholder dairy cows in the study area experienced prolonged CCI and CI, and thus, their reproductive performance was unsatisfactory. Moreover, the observed pre-weaning calf mortality rate is quite high. The serum PAG and P4 levels at the $4^{th}$ and the E1-S levels at approximately the $17^{th}$ week of pregnancy were well above the required levels for pregnancy testing in both Borana and crossbred cattle. Moreover, the observed serum profiles of PAG, E1-S, and P4 throughout gestation (and 10 weeks postpartum for PAG) were comparable to those reported for other breeds of cattle.

*Key words*: Endocrinology of pregnancy, pregnancy diagnosis/monitoring, reproductive/lactation performance, dairy cattle, Ethiopia.

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# Appendix

## Papers I–IV

This thesis is based on the following four papers, which will be referred to in the text by their respective Roman numerals:

I. Lobago, F., Bekana, M., Gustafsson, H., Beckers, J.F. & Kindahl H., 2006. Clinical features and hormonal profiles of cloprostenol-induced early abortions in heifers monitored by ultrasonography. *Acta veterinaria scandinavica 48*, 23 (open access). (http//www.actavetscand.com/content/48/1/23).

II. Lobago, F., Bekana, M., Gustafsson, H. & Kindahl, H., 2006. Reproductive performance of dairy cows in smallholder production system in Selalle, central Ethiopia. *Tropical Animal Health and Production 38*, 333–342.

III. Lobago, F., Bekana, M., Gustafsson, H. & Kindahl, H., 2007. Longitudinal observation on reproductive and lactation performances of smallholder crossbred dairy cattle in Fitche, Oromia region, central Ethiopia. *Tropical Animal Health and Production 39*, 395–403.

IV. Lobago, F., Bekana, M., Gustafsson, H., Beckers, J.F., Yohannes, G., Aster, Y. & Kindahl, H., 2007. Serum profiles of pregnancy-associated glycoprotein, estrone sulfate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. *Reproduction in Domestic Animals (In* press).

# Abbreviations

| | |
|---|---|
| AI | artificial insemination |
| BCS | body condition score |
| bPAG1 | bovine pregnancy-associated glycoprotein 1 |
| CADU | Chilalo Agricultural Development Unit |
| CCI | calving-to-conception interval |
| CI | calving interval |
| CSA | Central Statistics Authority |
| DALC | duration after last calving |
| EARO | Ethiopian Agricultural Research Organization |
| E1-S | oestrone sulphate |
| GDP | gross domestic product |
| HARC | Holetta Agricultural Research Centre |
| IAR | Institute of Agricultural Research |
| IBC | Institute of Biodiversity Conservation |
| ILCA | International Livestock Centre for Africa |
| MCLP | mixed crop and livestock production |
| OLA | onset of luteal activity |
| PAG | pregnancy-associated glycoprotein |
| P4 | progesterone |
| PG | prostaglandin $F_{2\alpha}$ |
| PSPB | pregnancy-specific protein B |
| SAS | Statistical Analysis System |
| SDDP | Selalle Smallholder Dairy Development Project |
| SPDDPP | Selalle Peasant Dairy Development Pilot Project |
| SUDP | small urban dairy production |

***To my:***

***Parents***

***Wife, Zelalem Zewdie***

***Son, Yeabsira***

***"More than any one who lived before me, I have been privileged to see more of the creator's miracles in Nature, which has been my greatest joy…"***

***Carl Linnaeus***

# Introduction

Agriculture (mainly crop and livestock production) is the mainstay of the Ethiopian economy, employing approximately 85% of the total population. Livestock production accounts for approximately 30% of the total agricultural GDP and 16% of national foreign currency earnings (IBC, 2004). Moreover, Ethiopia has diverse animal genetic resources and its relatively large livestock population (approximately 100 million) is well adapted to and distributed among diverse ecological conditions and management systems. The major farm animal genetic resources of the country include cattle, sheep, goats, donkeys, horses, mules, camels and chickens. As well, demand for livestock products in sub-Saharan Africa, and in developing countries in general, is likely to rise considerably as a result of rapidly growing human population, urbanization and income. This increase in demand for livestock products will have profound implications for food security, poverty alleviation, and the environment (Ehui, 2000).

In spite of the presence of large and diverse animal genetic resources, the productivity (i.e., meat and milk) of livestock remains low in many developing countries including Ethiopia, for various reasons, such as inadequate nutrition, poor genetic potential, inadequate animal health services, and other management-related problems. Consequently, crossbreeding of locally adapted cattle breeds with improved dairy cattle breeds has been adopted at various times by many developing countries in an effort to improve cattle productivity. However, the success of such crossbreeding programmes has varied depending on the several factors, including the simultaneous implementation of reasonable standards of animal nutrition, disease control, and husbandry and improved infrastructure status. The improvement of livestock production has been remarkable in many industrialized countries due to the integrated effect of rapid developments in several fields of the industry, such as breeding animals with superior genetic potential, increased feed production, improved animal health measures, and better husbandry methods (Bane and Hultnäs, 1977). In contrast, parallel improvements in livestock productivity have generally not occurred in developing countries probably due to lack of such integrated approaches. Thus, research could play a major role in improving our understanding, and discerning the opportunities and challenges a given sector is confronting, and subsequently creating sustainable options for overcoming the problems.

Crossbreeding of improved exotic dairy cattle breeds on a wide scale was introduced five decades back to upgrade the genetic potential of the indigenous zebu cattle, and subsequently to improve the dairy sector in Ethiopia. Since then, various efforts have been made to improve the dairy sector through artificial insemination or shared crossbred bull service or by distributing crossbred $F_1$ heifers particularly to the smallholder dairy farmers in urban and peri-urban areas and to those rural farmers located in close proximity to urban areas. Among the beneficiaries of the crossbreeding programme are the smallholder dairy farms located in the central highlands of Ethiopia, such as those in the Salalle area. However, animal performance depends not only on genetic merits, but also on other factors, such as nutrition, management, health, and environment. Thus, to ensure their success, crossbreeding programmes need to be monitored regularly, by evaluating both the reproductive and lactation performance of the crossbred cattle under the prevailing management and environmental conditions.

Farm data-recording systems at the smallholder production level are either totally absent or incomplete, so it is usually difficult to get reliable data with which to evaluate the performance of smallholder cattle. Moreover, owing to problems associated with logistics, high research costs, and other inconveniences, longitudinal observational studies are usually difficult to implement at the smallholder level. Consequently, in the past most research attempts have focused on institutional herds, and there has been only very limited efforts to make cross-sectional observations to evaluate the performance of dairy cattle in Ethiopia (for example, Swensson *et al*., 1981; Albero, 1983; Bekele, Kasali & Alemu, 1991; Mukasa-Mugerwa *et al*., 1991; Haile-Mariam *et al.*, 1993; Shiferaw *et al*., 2003). Interestingly, most of these limited research efforts have confirmed that the crossbred cattle have performed significantly better than indigenous zebu cattle have for the major lactation and reproductive traits considered. Moreover, observations made in some tropical countries have indicated that various factors such as genotype, location (district), season of calving, suckling status, parity number, and body condition score, have considerable effects on the performance of smallholder dairy cattle (Obese *et al.*, 1999; Msanga *et al*., 2000; Masama *et al*., 2003; Msanga and Bryant, 2003; Msangi, Bryant & Thorne, 2005). Thus, studies aiming at evaluating the performance of smallholder dairy cattle in Ethiopia, and of the factors influencing it, are vital.

Although most pregnancy failures occur due to embryonic/early foetal mortality up to 50 days post breeding, foetal mortality and subsequent abortion may occur afterwards to a lesser extent until the end of pregnancy (Ayalon, 1978; Forar, Gay & Hancock, 1995; Peters and Ball, 1995; Opsomer *et al*., 2007). Estimates of the frequency of endemic foetal loss ranged from 0.4 to 10.6% in 26 different studies of dairy herds conducted over five decades (Forar, Gay & Hancock, 1995). Consequently, accurately diagnosing and monitoring pregnancy status as early as possible after service to identify non-pregnant animals and subsequently take appropriate measures to rebreed them has a considerable effect on the reproductive performance of cattle. Rectal palpation, ultrasonography and hormone or pregnancy-specific antigen analysis –for example, analysis of progesterone (P4), pregnancy-associated glycoprotein (PAG), and oestrone sulphate (E1-S) –are the commonly employed techniques for diagnosing and monitoring pregnancy in cattle; however, all of these methods have their own advantages and disadvantages. Analysis of pregnancy-associated glycoprotein, produced by the trophoblastic cells of the foetus and specific to pregnancy, is a reliable indicator of viable pregnancy starting from the fourth week post service (Semambo *et al*., 1992; Szenci *et al*., 1998a).

It has been demonstrated that several management- and cow-related factors contribute to the occurrence of early pregnancy loss (Jousan, Drost & Hansen, 2005; Lopez-Gatius *et al*., 2002). Moreover, Lopez-Gatius *et al*. (2007) reported that some dam-, sire- and foetus-related factors influence PAG concentration in early pregnancy. Knowledge of the associations of pregnancy-related hormones or pregnancy-specific antigens profiles with various management- and animal-related factors throughout gestation is valuable for accurately diagnosing and monitoring pregnancy. Though rectal palpation is the only method employed for pregnancy diagnosis in Ethiopia, the introduction or wider application of other more up-to-date techniques (ultrasonography and hormone or pregnancy-specific antigen analysis) could be possible in the near future for research purposes and for pregnancy diagnostic purposes, at least in commercial large-scale dairy farms in Ethiopia. However, there is little or no information regarding the profiles of PAG, E1-S, or P4 during gestation in indigenous or crossbred cattle under existing environment and management conditions. Thus, it would be worthwhile to conduct research to establish the serum profiles of pregnancy-related hormones or pregnancy-specific antigens and to assess the effects of some factors that influence these profiles throughout the gestation period in indigenous zebu and crossbred cattle.

# General background

## Dairy cattle production in Ethiopia

The latest estimate of Ethiopia's cattle population is approximately 44.3 million (CSA, 2004); these are predominantly indigenous zebu cattle, which are well adapted to and distributed among the diverse ecological conditions and management systems of the country. Though no exhaustive identification and characterization work has been conducted, it is suggested that there are over 25 types/breeds of indigenous cattle, the most popular ones including Borana, Horro, Fogera, Arussi, Karayu and Nuer (IBC, 2004). The existing dairy cattle production systems in Ethiopia belong to either of the following four major livestock production systems: rural smallholder (mixed crop–livestock) production, pastoral and agro–pastoral production, urban and peri-urban smallholder dairy production, and specialized commercial dairy production systems.

### *The rural smallholder (mixed crop–livestock) production system*

This system predominantly exists in the highland agro–ecological zone, where the climate favours both crop cultivation and livestock rearing as complementary enterprises. In this system, all major types of farm animals except camels are found and farmers usually prefer to keep mixtures of farm animal species. Approximately 80% of Ethiopia's population is settled in the highlands (above 1500 meters above sea level), which represent 45% of the total land (IBC, 2004). The highlands are more densely populated and subjected to more overgrazing and natural resource degradation than are the lowlands. This system is characterized by land scarcity and the major livestock feed resources include grazing on marginal lands, crop aftermath, and crop residues. Moreover, dung is an important source of fuel in this system. The smallholder farmers in this system predominantly raise indigenous zebu cattle breeds and farming is subsistence in nature. However, some farmers located near urban centres and that having access to milk markets for selling surplus milk own crossbred cattle.

### *Pastoral and agro–pastoral production system*

This system exists in the vast lowland areas of the country where arid and semi-arid agro–climates dominate. This system features sparsely populated pastoral rangelands, where subsistence is mainly based on livestock and livestock products except in agro–pastoral areas, where some crops are produced for both subsistence and the market. The livestock husbandry in this system is dominated by goats, cattle, sheep, and camels. The main source of food is milk, so pastoralists tend to keep large herds to ensure sufficient milk supply and income (IBC, 2004).

### *Urban and peri-urban smallholder dairy production system*

In this production system, the dairy cattle production is either a full time or a part time business. These smallholder dairy farms predominantly keep a small number of crossbred cows in a zero-grazing system to produce milk for both home consumption and sale. Animals are fed with natural grass hay and crop residue and supplemented with various amounts of agro–industrial by-products. Given suitable government policy support and access to markets and services, there is great potential to develop smallholder dairy schemes in peri-urban and urban areas (IBC, 2004).

*Specialized commercial dairy farms*

Commercial dairy farms are located in urban and peri-urban areas mainly in and around the major cities and produce milk for sale. These farms are specialized dairy farms that own either crossbred and/or pure exotic breeds of dairy cattle. The commercial farms are small- to large-scale dairy farms, the large-scale farms being concentrated in and around the capital.

Based on the type and quantity of inputs, and on the objective and level of intensity of production, the major livestock production systems presented above could be further classified as low-, medium-, and high-input production systems. More than 95% of the livestock population in Ethiopia is kept in low-input systems, in which production is fully dependent on natural resources and the demand for inputs is limited. Most cattle raised in the low-input systems are indigenous breeds (IBC, 2004).

## Dairy cattle crossbreeding programmes in Ethiopia

The prevailing heat stress, external and internal parasites, infectious disease, poor nutrition, and other concurrent problems, impedes the introduction and use of selected, high-producing exotic breeds in most tropical and subtropical countries. Consequently, the most suitable cows for dairy production in these areas are ones with a proportion of genes from high-producing cattle of temperate origin and a proportion from well-adapted but low-producing cattle, in many cases a *Bos taurus* × *Bos indicus* cross, often with the advantages of heterosis (Mason, 1974; Albero, 1983).

In Ethiopia, the crossbreeding of improved European with indigenous zebu cattle breeds was introduced in the 1930's by Italians. However, wide-scale cattle crossbreeding activities were started later by the Institute of Agricultural Research (IAR) and Chilalo Agricultural Development Unit (CADU) using Holstein-Friesian, Jersey, and Simmental sires that were crossed with the local Horro, Borana and Arussi dams (Brännäng *et al*., 1980; EARO, 2001). Since then, governmental and non-governmental organizations have made various efforts to improve the dairy sector by establishing dairy cattle improvement ranches and distributing crossbred $F_1$ heifers to smallholder farmers (EARO, 2001; Kelay, 2002). For example, in 1987 a project known as the Selalle Peasant Dairy Development Pilot Project (SPDDPP) was established and introduced crossbred dairy cattle and improved management skills in the highlands of Ethiopia. This pilot project was later continued with a new project called the Selalle Smallholder Dairy Development Project (SDDP), which had the same objectives but a broader range of activities encompassing other parts of the country (Kelay, 2002).

## Reproductive and lactation performance of dairy cattle

The reproductive efficiency of a dairy herd can be measured in several ways, such as by measuring pregnancy rate, percentage of cows calving each year, average calving interval, average number of days dry, and number of live calves born each year. Although each of these measures affects the profitability of the dairy business in a slightly different way, the calving interval affects both the total milk production of the dairy herd and the number of calves born. In most modern dairies, the general practice is to breed cows early, with the aim of establishing a calving interval of 12 to 13 months, which is considered optimum; hence, calving interval is considered an important index of reproductive performance (Roberts, 1986; Arbel *et al*., 2001).

Calving interval (CI) has two components: 1) calving-to-conception interval (CCI) or days open, which is considered to be the most important component determining the length of the calving interval, and 2) gestation length, which is more or less constant, varying slightly due to breed, calf sex, litter size, dam age, year, and month of calving, and little can be done to significantly manipulate the gestation length (Mukasa-Mugerwa *et al*., 1991). The CCI itself is influenced by cow and management/environment-related factors, such as method and efficiency of heat detection, type and efficiency of breeding service and the ability of the cow to resume regular ovarian cyclicity after calving, display an overt heat signs, and conceive with the given service.

Milk yield and reproductive efficiency play major roles in determining the profitability of a dairy herd (Britt, 1985; Arbel *et al*., 2001). Inadequate reproductive performance is one of the most costly problems facing dairy producers. For example, Esslemont and Kossaibati (1997) found that poor fertility remained the single most important reason for involuntary culling, whatever the age of the animal, based on investigations of 50 dairy herds in England. In addition, Pelissier (1982) estimated the total losses for dairy farmers and consumers associated with sub-optimal reproductive performance in the US dairy industry, accounting all major sources of inefficiency to be $1.2 billion or approximately $116 per cow. Moreover, Strandberg and Oltenacu (1989) demonstrated that the effect on net return per year of delaying pregnancy by one day ranged from SEK 0.3 to 11.6 depending on calving month, stage of lactation, parity, and production level and suggested that for all combinations of characteristics early conception was the most profitable.

The lactation performance of dairy cattle is usually measured by determining the total milk yield per lactation or per year, average daily milk yield, lactation length, lactation persistency, and milk composition. Generally, the reproductive performance and lactation performance of dairy cattle are closely associated with each other. Breeding failure has a clear negative influence on milk production and farm income and determines the future sustainability of a dairy farming operation. Milk production level and lactation persistency are crucial factors determining the appropriate calving interval (Arbel *et al*., 2001). On the other hand, the costs of fertility depend on the stage of lactation and the shape of the lactation curve. Cows normally have a lactation curve that loses 8 to 10% per month after the peak, but those rare animals whose production declines by only 4% or so may make a longer calving interval justified (Esslemont, 2003).

In most modern dairy farms, a lactation length of 305 days is commonly accepted as a standard. However, such a standard lactation length might not work for smallholder dairy cows in which the lactation length is extended considerably in most cases (Teodoro and Madalena, 2003; Masama *et al*., 2003; Msangi, Bryant & Thorne, 2005). Furthermore, such an extended lactation period has practical significance for the smallholder dairy farmer as it provides compensation for the usually extended calving interval (Tanner, McCarthy & Omore, 1998). The profitability of short or extended lactation length depends on various factors, including the lactation length persistency. Numerous studies have documented that additional days in which cows are not pregnant beyond the optimal time post calving are costly (Groenendaal, Galligan &Mulder, 2004; Meadows, Rajala-Schultz &Frazer, 2005). However, Borman, Macmillan & Fahey (2004) demonstrated that extended lactations are suitable for some dairy enterprises and that the suitability depends particularly on cow milk potential, the ability to grow pasture or feed supplements economically, management expertise, environmental constraints, herd size and labour availability. Moreover, Österman and Bertilsson (2003) suggested that by combining a longer calving interval with increased milking frequency, daily milk production from one calving to another could be increased, making an increased calving interval an interesting option for dairy farmers. In addition, an economic advantage in extending lactations (by 60 days) was found even in the case of high-yielding cows. This advantage was greater for primiparous cows, because of the high persistency of their milk production and the increase in the fat and protein contents of their milk as lactation progresses (Arbel *et al*., 2001). Thus, it is of interest to properly evaluate the economic benefits and subsequently optimize both the lactation length and calving interval under the given production level and prevailing management conditions.

## Factors influencing the reproductive and lactation performance of smallholder dairy cattle

The performance of animals depends not only on their genetic merits, but also on other factors such as nutrition, management, health, and environment. Many factors influence the reproductive performance of lactating dairy cows. Management factors such as accuracy of heat detection, use of proper inseminating techniques, proper semen handling, and appropriate herd health policies can directly influence the reproductive performance of a dairy herd. In addition other factors beyond the immediate control of management may impact fertility; these factors include milk production of the cow, age of the cow, and season of year (Hillers *et al.*, 1984). A variety of environmental factors affect the onset of ovarian cycles in the postpartum period and the most important of these are suckling, milk yield, nutritional status, and season (Peters, 1984). Swensson *et al.* (1981) suggested that malnutrition, disease, milk let-down interference, weak heat symptoms, and inbreeding are factors that commonly result in very low fertility in unimproved breeds.

Msangi, Bryant & Thorne (2005) did a longitudinal study in Tanzania to examine factors influencing milk yield in smallholder crossbred cows. They investigated the effects of location (district), calving season, body condition score (BCS) at calving, calving year, herd size, source of labour (hired or family labour), calf-rearing method (bucket-fed or partial suckling), and parity number, and found that calving year, calf-rearing method and BCS significantly influenced the daily milk yield. This group (Msangi, Bryant & Thorne, 2005) demonstrated that milk production was mainly influenced by BCS at calving, at which time the lactation milk yield increased quadratically from score 1 to 3; they concluded that BCS at calving may provide a simple single indicator of the nutritional status of a cow. In addition, Muraguri, Mcleod & Taylor (2004) from Kenya reported that commercial concentrate supplementary feeding of lactating smallholder cows led to a significantly higher mean daily milk yield than that of non-supplemented ones throughout the year (18.6% higher annual milk off-take).

With respect to effect of breed, it has been found that crossbreeding has improved the age at first calving and oestrous manifestation of crossbred cows, compared with the local ones, kept under equal and satisfactory feeding, management, and health-control regimes (Swensson *et al*., 1981). Moreover, Albero (1983) showed that in 14 small dairy farm cooperatives located in the central highlands of Ethiopia, $F_1$ heifers performed significantly better than did zebu (CIs of 371 versus 421 days and total milk yield per lactation of 2013 versus 429 kg, respectively). However, a decline in both the productive and reproductive performance with increasing fractions of *Bos taurus* above the $F_1$ crosses was reported in medium-/low-input production systems (Madalena *et al*., 1990).

In smallholder dairy production systems, reports vary among the different investigators with regard to the effects of certain factors on the reproductive and lactation performance. For example, Msanga and Bryant (2004) found no difference in the productivity of cows, measured as annual milk off-take, between those that suckled their calves and those whose calves were bucket reared and whose calf weights were similar at one year of age. On the other hand, Msanga and Bryant (2003) observed an early peak lactation yield in bucket-rearing cows, while suckling cows had a flatter but more persistent (sustained) curve; the lactation persistency was maintained to a significantly greater extent in suckled than in bucket-rearing cows. Moreover, Msanga *et al*. (2000) from Tanzania reported that the lactation yields of smallholder dairy cows were significantly influenced by factors such as year of calving, district, proportion of Holstein genes, and herd size, while lactation length was significantly affected by district and herd size. With respect to reproductive performance, Msanga and Bryant (2003) found no significant differences in the interval from calving to first oestrus, insemination and conception between two crossbred genotypes (60–80% and >80% *Bos taurus* inheritance), between cows whose calves were reared by suckling or bucket. In contrast, Obese *et al*. (1999) from Ghana reported that calving interval was significantly influenced by factors such as location, season of calving, parity, and BCS in smallholder dairy herds.

## The significance of diagnosing and monitoring early pregnancy

Reproductive failure is one of the major factors affecting output in beef and dairy herds. It is generally accepted that fertilization rates after natural service or artificial insemination are close to 90%, while calving rates to a single insemination are close to 50% (Diskin and Sreenan, 1980). It has been demonstrated that most embryonic loss occurs between days 8 and 16 post insemination and that the embryo survival rates up to 8 days was 93%; however, the survival declined markedly to 66% and 58% by days 16 and 42 post insemination, respectively, in beef heifers (Diskin and Sreenan, 1980). Surprisingly, much lower conception rate (29.3%) on days 60–66 after AI and quite high rate of pregnancy loss (18.6%) between days 25–32 and 60–66 after AI were reported in lactating Holstein cows recently (Sartori *et al.*, 2006). Thus, accurately diagnosing and monitoring pregnancy status as early as possible after service and detection of non-pregnant animals before the first expected return to oestrus 18 to 24 days after service would be ideal.

On the other hand, manipulating the pre-implantation embryo, particularly in the sheep and cow, can severely influence foetal growth resulting in unusually large offspring. The phenomenon of the production of large offspring after embryo manipulation opened up new perspectives on the roles of reproductive technology in both livestock and human reproduction (Walker, Hartwich & Seamark, 1996). Consequently, monitoring embryo/foetal wellbeing is of increasing interest if we are to characterize and better understand the phenomena of large-offspring syndrome and associated abnormalities, including increased rates of abortion and physical abnormalities, increased gestation length and increased levels of mortality and morbidity (Walker, Hartwich & Seamark, 1996). In this regard, various ultrasonographic investigations have been made to evaluate the bovine foetus by monitoring foetal heart rate and foetal growth parameters (Pierson and Ginther, 1984; Kastelic *et al.*, 1988; Bertolini *et al.*, 2002; Breukelman *et al.*, 2004, 2005). In humans, monitoring the foetal heart rate in early pregnancy was found to be a good predictor of foetal viability (Laboda, Estroff & Benacerraf, 1989; Schats, Jansen & Wladimiroff, 1990).

## Methods for diagnosing and monitoring pregnancy

The most popular methods currently employed for early pregnancy diagnosis in cattle are rectal palpation, ultrasonography and hormone or pregnancy-specific antigen analysis (mainly progesterone, pregnancy-specific antigens, and oestrone sulphate). However, all these pregnancy diagnostic techniques have their own advantages and disadvantages with regard to the time interval of diagnosis after service, diagnosis accuracy and efficiency, and effect on embryonic/foetal losses.

### *Rectal palpation*

Manual transrectal palpation of the reproductive tract approximately 35 days post service is commonly used to determine pregnancy status (Roberts, 1986; Franco *et al*., 1987; Pieterse *et al.*, 1990). The accuracy of this method depends on the experience of the operator and on the criteria that are used: fluctuation of the uterus as a result of the presence of foetal fluids, identification of the amniotic vesicle, and slipping of the chorio–allantoic membrane. However, it has been suggested that a positive diagnosis of pregnancy can be made as early as 27 days post service in some animals by palpating and identifying the amniotic vesicle (Zemjanis, 1970; Paisley, Mickelsen & Frost, 1978). In addition, pregnancies of greater than 45 days' duration can be determined by detecting the foetal membrane "slip", cotyledons, or the foetus itself (Zemjanis, 1970; Paisley, Mickelsen & Frost, 1978).

Rectal palpation has also been performed very early to predict the outcomes of pregnancy. Paisley, Mickelsen & Frost (1978) suggested that upon rectal palpation, finding a quiescent uterus and a fully developed corpus luteum in an animal serviced 20–22 days previously is highly suggestive that it has conceived; they suggest this method could be 85 to 90% accurate at predicting the outcome of breeding. The more criteria included in the pregnancy diagnosis, the greater the accuracy; however, such accuracy is accompanied by a significant increase in embryo/foetal mortality (Franco *et al*., 1987; Pieterse *et al*., 1990). Based on breeding and calving records, palpation per rectum after day 35 post insemination proved to be 99% accurate in diagnosing pregnancy and non-pregnancy (Badtram *et al*., 1991).

One of the commonly indicated disadvantages of rectal palpation is the risk of inducing embryo/foetal loss. However, the effect of manual rectal palpation on the incidence rates of induced embryo/foetal losses varies according to different reports. For example, the rate of induced foetal death due to pregnancy diagnosis by rectal palpation per rectum between days 42 and 46 was estimated to be 11.8% based on milk P4 content at day 63, or 9.5% based on palpation per rectum at day 90 (Franco *et al*., 1987). Moreover, it was observed that there were significant differences between palpators in the induction of foetal attrition (Abbitt *et al*., 1978; Franco *et al*., 1987). On the other hand, the incidence of prenatal mortality following pregnancy diagnosis by rectal palpation (done by clinicians and senior veterinary students) was 5.8%, 6.0%, and 0.8% for 802 pregnancies diagnosed at less than 35 days, between 35–45 days and over 45 days, respectively (Paisley, Mickelsen & Frost, 1978). The authors of this last work concluded that multiple palpations may contribute to foetal losses, but that the incidence was not high enough to justify changing technique or having veterinary students avoid palpation.

### *Ultrasonography*

Ultrasonic imaging technology allows non-invasive visualization of the reproductive tract and has allowed the study of dynamic interactions (Pierson and Ginther, 1988). Accurate pregnancy diagnosis could be achieved based on the recognition of a proper embryo with a beating heart, at between 26 and 34 days, by using ultrasonography in cattle (Pierson and Ginther, 1984; Pieterse *et al*., 1990).

Under controlled condition in heifers, the conceptus (embryonic vesicle) was first detected as a discrete nonechogenic (black) oblong structure in the uterine horn ipsilateral to the corpus luteum between 12 to 14 days post ovulation using a high-quality diagnostic ultrasound (Pierson and Ginther, 1984). Kastelic *et al*., (1988) reported that the first detection of the embryo proper and heartbeat was possible as of 21.4 days, on average (range 19–27 days), post ovulation in 58 heifers.

A considerable difference was noted between the reliability of ultrasound examinations performed under field conditions at an early stage (days 21 to 25) and of those performed at a later stage (days 26–33). The sensitivity and specificity of the ultrasound examinations between days 21 and 25 were 44.8% and 82.3%, respectively, but between days 26 and 33 the results were 97.7% and 87.8%, respectively (Pieterse *et al*., 1990). On the other hand, Badtram *et al*. (1991) reported that the diagnostic accuracy and sensitivity of ultrasound examinations carried out by two operators were 50% and 25% between days 16 and 22 post insemination, versus 70.2% and 68.8% between days 23 and 31, respectively. These authors concluded that pregnancy diagnosis in the cow before 30 days post service using a real-time B-mode ultrasound with a 5-MHz probe was not highly accurate under field conditions.

On the other hand, one of the most profound clinical and research applications of ultrasonography in cattle involves the detecting embryonic death and monitoring the physical events preceding and following it (Pierson and Ginther, 1984; Kastelic *et al*., 1988). Kastelic *et al*. (1988) observed an apparent reduction in embryonic heart rate prior to embryonic death in heifers. They suggested that indications of embryonic distress or of impending death can be obtained by monitoring embryonic heartbeat, which will also allow, for research purposes, determination of the precise time of death of the embryo following a given treatment. Thus, diagnostic ultrasound is a powerful tool for monitoring embryo/foetal viability in cattle.

### *Hormone or pregnancy-specific antigen assay*

Determining the milk progesterone level has been accepted as a reliable means of pregnancy diagnosis from 21 to 24 days post service in the cow, and its accuracy is reported to be 80% and 100% for the diagnosis of pregnant and non-pregnant animals, respectively (Heap *et al.*, 1976; Franco *et al*., 1987). Milk progesterone levels that remain elevated throughout the period 18–24 days post service is presumptive evidence of pregnancy, and the overall accuracy of milk progesterone testing for pregnancy detection is reported to be 86% (Hoffmann *et al*., 1974). Progesterone secretion is not specific to pregnancy, so the accuracy of testing for it is influenced by various factors. For example, pathological conditions such as pyometra, segmental aplasia of the mullerian duct system, luteal cysts, embryonic or foetal death, and the presence of macerated or mummified foetus can result in the maintenance of an active corpus luteum (persistent corpus luteum) and elevated milk or plasma progesterone levels that are suggestive of pregnancy.

The development of methods for isolating and characterizing pregnancy-specific protein B (PSPB) (Butler *et al*., 1982) and bovine pregnancy-associated glycoprotein 1 (bPAG1) (Zoli *et al*., 1991), and the later development of the corresponding RIA technique for these proteins (for PSPB, Sasser *et al*., 1986; and for bPAG1, Zoli *et al*., 1992), have opened a door for the reliable diagnosis of pregnancy in cattle using pregnancy-specific antigens. Thus, pregnancy-associated glycoprotein (PAG), or bovine pregnancy-associated glycoprotein 1 (bPAG1), which is similar with pregnancy-specific protein B (PSPB) (Xie *et al*., 1991; Zoli *et al*., 1991, 1992), is produced by the trophoblastic cells of the foetus and its secretion is specific to pregnancy. Both PAG/bPAG1 and PSPB are inactive members of the aspartic acid proteinase family and are identical in gene nucleotide sequence but differ in carbohydrate and sialic acid content (Xie *et al*., 1991; Lynch, Alexander & Sasser, 1992). In bovine, PAG or PSPB is used as early marker of pregnancy and for monitoring ongoing pregnancies (Sasser *et al*., 1986; Zoli *et al*., 1992). Pregnancy-associated glycoprotein, or PSPB is detectable as of approximately 24 days post conception; it reaches its peak level at approximately the time of parturition and drops after calving, but is still detectable for up to three months due to its long half-life in postpartum cows (Sasser *et al*., 1986; Humblot *et al*., 1988a; Zoli *et al*., 1992).

Zoli *et al*. (1992) reported a 95% overall accuracy of the PAG radioimmunoassay as of 35 days post service, with accuracies of 93% and 98% for detecting pregnant and non-pregnant cattle, respectively. Moreover, Szenci *et al*. (1998a) recorded sensitivities of 95% and 100% at 29 or 30 and 44 days post service, respectively. Similarly, a report from a field investigation of 94 dairy farms in Scotland indicated that the bPAG radioimmunoassay had an overall efficiency of 98% (sensitivity and specificity of 100% and 93%, respectively) in routine pregnancy diagnosis (Skinner *et al*., 1996). However, the long half-life of PSPB or PAG, which causes the slow decline in its concentration in the maternal serum after embryonic/foetal death (Humblot *et al*., 1988b; Semambo *et al.,* 1992; Szenci *et al*., 1998a) and in the early postpartum period (Sasser *et al*., 1986; Zoli *et al*., 1992; Kiracofe *et al*., 1993), is currently the main drawback of the PAG assay as a pregnancy diagnostic test. Thus, further detailed research is a crucial if we are to solve the problems of long half-life and carry-over antigen from previous pregnancy in the early postpartum period and subsequently improve the overall accuracy of the PAG assay as a routine pregnancy diagnostic test.

## Status and prospects of pregnancy diagnosis in Ethiopia

At present, rectal palpation is the only practical routine diagnostic method used for pregnancy determination in dairy farms in Ethiopia; it is usually carried out late, after 60 days post service, by AI technicians or other animal health professionals. Based on personal observation in the field, there is variation in the experience and confidence of AI technicians with regard to pregnancy diagnosis. Moreover, most of the AI technicians received only very short initial training and never get refreshment training and there are no follow-up systems to check their field performance once they have started to work. Thus, problems with accurate and timely pregnancy diagnosis could be contributing to the commonly extended calving interval observed in dairy farms in Ethiopia (**Paper II**).

Considering the high cost, skilled personnel requirements, and other related inconveniences associated with the latest pregnancy diagnostic methods (i.e., ultrasonography and hormone or pregnancy-specific antigen assay), manual rectal palpation could be an ideal technique for routine pregnancy diagnosis in dairy farms in Ethiopia. It seems that rectal palpation could well continue to be the only routine pregnancy diagnostic tool, at least at the smallholder-dairy-farm level, in the coming one or more decades: it has the advantages of only requiring labour input, which is cheap and is highly accurate after 45 days post service. Thus, it is worthwhile to upgrade the skills of existing AI technicians and other animal health professionals involved in the dairy sector; the training manual (curriculum) for AI technicians should be revised, to equip them with the necessary skill to enable them to diagnose pregnancy as early as 40 days post service. Meanwhile, other more up-to-date pregnancy diagnostic methods, such as hormone or pregnancy-specific antigen analysis and ultrasonography, could be employed at wider scope for research purposes. Moreover, these methods could possibly be used for pregnancy diagnosis in the future at least in commercial dairy farms in Ethiopia. In particular, diagnostic ultrasonography could be used as a valuable tool for confirming early pregnancy during the practical training of AI technicians and veterinary students, to support them in building their rectal palpation skill for early pregnancy diagnosis.

# Aims of the thesis

The general aim of this thesis was to evaluate the reproductive and lactation performance of smallholder dairy cattle in the central highlands of Ethiopia with particular emphasis on diagnosing and monitoring pregnancy.

## The specific aims were:

- To describe the clinical features and plasma profiles of PAG, PG metabolite and P4 in heifers after cloprostenol-induced early abortions
- To investigate the reproductive and lactation performance of smallholder dairy cattle
- To assess the effects of certain factors that influence the reproductive and lactation performance of smallholder dairy cattle
- To establish the serum profiles of PAG, E1-S, and P4 throughout pregnancy in Ethiopian Borana and crossbred cattle
- To evaluate the effects of certain dam- and foetus-related factors on the serum profiles of PAG, E1-S, and P4

# Methodological considerations

## Study area

The first experiment (**Paper I**) was carried out at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala. Two of the studies (**Papers II** and **III**) were conducted in the Selalle area of the Oromia region (North West Shoa zone), situated in the central highlands of Ethiopia. The Selalle area is located between 25 and 150 km northwest of the capital, Addis Ababa (Fig. 1) and lies on an elevated plateau ranging from 2400 to 3500 meters above sea level. The average annual temperature and rainfall are 15–18ºC and 1000–1500 mm, respectively. The fourth study (**Paper IV**) was carried out in Holetta Agricultural Research Centre (HARC), which is located in the central highlands of Ethiopia some 40 km west of Addis Ababa (Fig. 1). The Holetta area receives an average annual rainfall of 1200 mm and experiences minimum and maximum temperatures ranging from 2–9ºC and 20–27ºC, respectively.

## Study animals and management

As an introductory study (**Paper I**), four Swedish Red and White Breed (SRB) heifers were used. The heifers were fed according to Swedish standards and the experiment was done, with the approval of the local ethics committee, at the Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala. A randomly selected 300 smallholder dairy farms that belong to five districts of Selalle area (60 farms from each district) were included in the cross-sectional study (**Paper II**). Based on the previous classification of dairy production systems (ILCA, 1994), the studied smallholder dairy farms were categorized as either small urban dairy production (SUDP) or mixed crop and livestock production (MCLP) system. The smallholder dairy farms categorized in the SUDP system were those located in towns of each of the five districts, while those farms located in the rural villages of the respective districts were included in the MCLP category. Twenty-four randomly selected smallholder dairy farms located in Fitche town, Selalle zone that belong to the SUDP system were used for the longitudinal observational study (**Paper III**). The studied farms had an average herd size of 6.8 cattle, including a total of 69 crossbred cows. Fifty-four cows and heifers (26 Borana and 28 crossbred) owned by HARC were employed for the fourth study (**Paper IV**). The animals kept by HARC were allowed to graze on natural pasture for an average of seven hours in the daytime and supplemented with either native hay and/or other roughage at night; moreover, cows were given concentrate feed during late pregnancy and lactation.

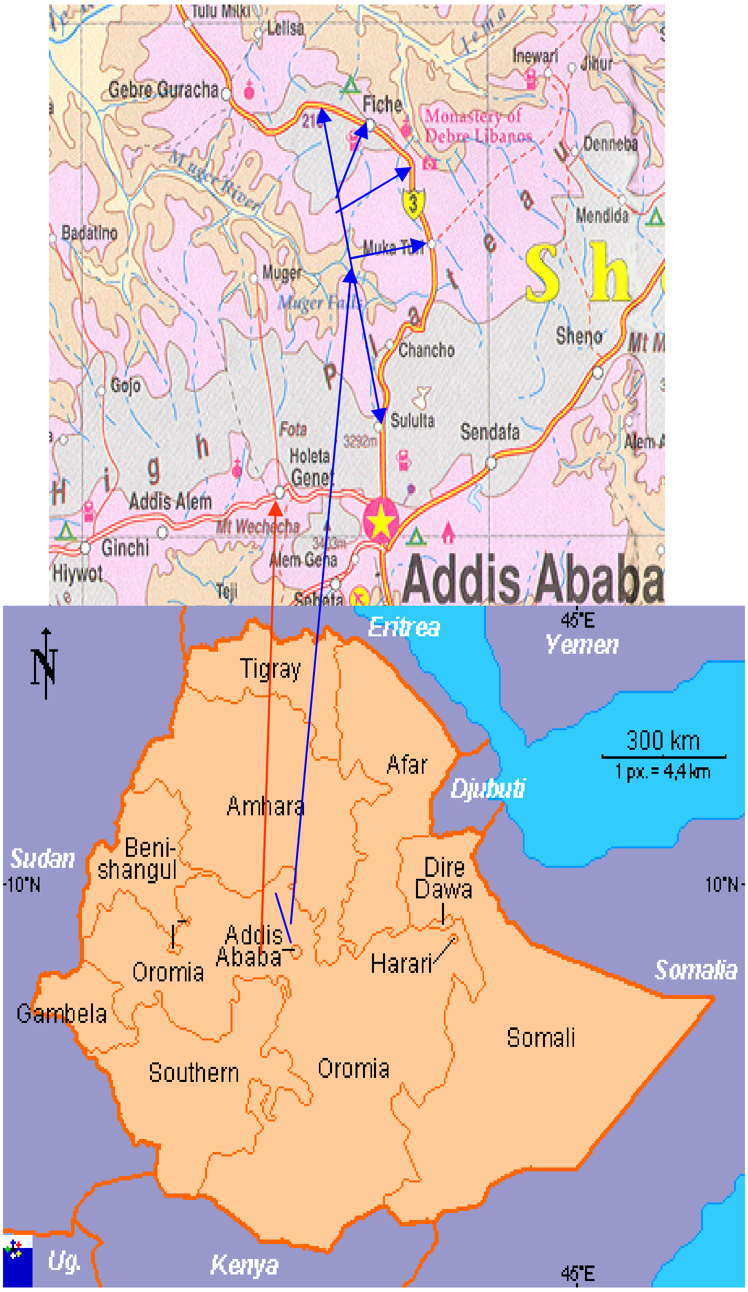


Figure 1. Map of Ethiopia (bottom) and part of the central highlands of Ethiopia (top) indicating the study sites-Selalle area (blue arrows) and Holetta (red arrow).

## Study design

For the introductory investigation (**Paper I**), a transrectal ultrasonography was employed to confirm pregnancy and monitor embryo/foetal viability weekly until 7 days before cloprostenol treatment and then daily until foetal death was confirmed. Heifers were given one intramuscular injection of 500 µg of cloprostenol (Estrumate®). The four heifers (1, 2, 3, and 4) were at day 63, 77, 83, and 116 of pregnancy, respectively, on the day of cloprostenol injection. Blood samples were collected from the heifers starting one week before to nine days after cloprostenol treatment. The plasma samples were analysed for PAG, prostaglandin $F_{2\alpha}$ (PG metabolite), and P4 using radioimmunoassay methods, following the procedures described by Zoli *et al.* (1992), Granström and Kindahl (1982), and Forsberg *et al.* (1993), respectively.

For the cross-sectional study (**Paper II**), the selected smallholder farms were visited once (twice in a few cases to confirm doubtful pregnancies) and data on the reproductive histories of cows since their last calving and on herd management were collected by examining records, interviewing farm owners and rectal examinations of individual animals. The body condition score (BCS) was determined using a nine-point scale (from 1 representing lean minus, to 9 representing fat plus) (Nicholson and Butterworth, 1986). The data obtained from the reproductive, breeding, and management histories of 300 herds (comprising 940 cows) were used to evaluate the reproductive performance of the dairy herds. The variable duration after last calving (DALC), which refers to the number of days from last calving to the day of examination was used to assess the reproductive performance of non-pregnant cows. Only those non-pregnant cows for which 60 or more days had elapsed since last calving were considered.

In the longitudinal study (**Paper III**), the selected farms were regularly visited once or twice per week; reproductive-, breeding-, health-, lactation- and management-related data were recorded for two years and the obtained data were analysed. Milk samples were collected weekly from 43 postpartum cows starting 8–12 days post calving to a maximum of 90 days after calving. The milk P4 level was analysed using an ELISA kit (Ovucheck) and used to determine the onset of luteal activity (OLA) after calving. Moreover, the daily milk yields of cows were recorded twice per week and used to estimate the average daily milk yield of a given lactation week.

The fourth study (**Paper IV**) involved two groups of pregnant animals: early pregnant (19 Borana and 19 cross) and late pregnant (7 Borana and 9 cross) animals. Transrectal ultrasonography was employed to confirm pregnancy (between 28 and 35 days post breeding) and to monitor embryo/foetal viability (every two weeks until the 13$^{th}$ week of pregnancy) in the early pregnant group. Blood samples were collected weekly from the 4$^{th}$ to the 13$^{th}$ week and then monthly up to the 33$^{rd}$ week of gestation in the early pregnant group; in the late pregnant group, blood was sampled weekly starting from 36 weeks of pregnancy until parturition, and thereafter every two weeks up to 10 weeks post calving. Blood samples were analysed for PAG and P4 using radioimmunoassay, while

ELISA was employed for the E1-S assay. The hormones and PAG analyses were carried out at the Department of Physiology of Animal Reproduction, Faculty of Veterinary Medicine, University of Liège, Belgium.

## Statistical analyses

The data on calving interval (CI), calving-to-conception interval (CCI), duration after last calving (DALC), first observed oestrus, and body condition score (BCS) were evaluated using the General Linear Model (Proc GLM) (**Papers II** and **III**), while the data on milk yield (**Paper III**) and serum PAG, E1-S and P4 concentrations (**Paper IV**) were evaluated using the repeated measures statement of the MIXED procedure (Proc MIXED) (SAS Institute Inc., Cary, NC, USA). The model included the fixed effects of location (district), production system, suckling status, and exotic gene percentage (**Paper II**); suckling status, parity number, and calving season (**Paper III**); and pregnancy stage (week), breed, parity, dam body weight, foetal sex, and foetal birth weight (**Paper IV**). Moreover, the individual cow was considered a random factor in the MIXED model (**Papers III** and **IV**). The data on CCI and DALC (**Paper II**) were transformed to natural logarithms before analysis. The chi-squared test was used to assess the fixed effects of the factors on the prevalence of pre-weaning calf mortality (**Paper II**).

The milk yield data for the first 14 and the following 15–43 lactation weeks were analysed separately **(Paper III)**. The P4 concentrations from four to 13 weeks post service (early gestation) and from 17 weeks post service to parturition (late gestation) were analysed separately, while PAG was only analysed in early gestation (4 to 13 weeks post service). For E1-S, the concentration from 17 to 33 weeks post service (mid gestation) and from 37 weeks post service to parturition (late gestation or prepartum) were analysed separately (**Paper IV**). Pearson correlation coefficients (PROC CORR) were employed to test the associations among PAG, P4, and E1-S (**Paper IV**). The means of the different groups in both models used were separated using Tukey's studentized range test (**Papers II, III** and **IV**). A non-parametric survival analysis method (Cox Proportional Hazard Regression, SAS Institute) was employed to determine the fixed effects of suckling status, parity number, calving season and milk yield on the binary outcome of the first onset of luteal activity (OLA) for the first 90 days after calving (**Paper III**). Differences were considered to be statistically significant at $P < 0.05$ (**Papers II, III** and **IV**). The half-life of PAG (**Paper I**) was estimated using the following formula (Kiracofe *et al.*, 1993; Szenci *et al.*, 2003): $T_{1/2} = [\ln(C/.5C)]/\lambda$, where C is the plasma concentration of PAG at time zero and $\lambda$ is the slope of the regression equation.

# Major findings

## Clinical features and profiles of pregnancy-associated glycoprotein, prostaglandin $F_{2\alpha}$ metabolite and progesterone in association with abortion

The foetal heartbeat rates ranged from 170 to 186 beats per minute for all foetuses up to the date of cloprostenol injection (**Paper I**). Foetal deaths were confirmed within two days of cloprostenol treatment. Two of the heifers (at days 63 and 77 of gestation) had thick mucous vaginal discharge and displayed signs of standing oestrus, whereas the remaining two (at days 83 and 116 of gestation) had blood-tinged discharge on the second day post treatment. The plasma concentrations of PAG, PG metabolite, and P4 were 8.4–40.0 ng/mL, 158–275 pmol/L, and 20.7–46.9 nmol/L, respectively, prior to cloprostenol treatment. After the foetus was expelled, the concentrations of PAG began to decrease gradually with an estimated half-life of 1.8–6.6 days. The plasma level of PG metabolite began to display short lasting peaks within three hours of cloprostenol injection, while plasma P4 concentrations dropped sharply to less than 4 nmol/L within 24 hours of the treatment. In the early pregnant group followed (**Paper IV**), a Borana cow experienced spontaneous abortion approximately 10 weeks post conception; its serum concentrations of PAG started to decline gradually after 10 weeks post conception but those of P4 dropped sharply.

## Dairy herd type, composition, and management and body condition score (Paper II)

The smallholder dairy herds in the Selalle area comprised indigenous zebu and crossbred (zebu × Holstein-Friesian/Jersey) cattle. Of 940 cows examined, 383 (40.8%), 491 (52.2%), and 66 (7%) were pregnant, non-pregnant, and pregnancy uncertain, respectively, at the day of examination. All dairy herds in the MCLP system were allowed to graze on natural native pasture, whereas only approximately 16% of the farms in the SUDP system practiced semi-grazing on natural pasture. Breeding was done either by natural mating using a shared bull or by AI or both. Approximately 36% of the farms in the MCLP used bull service only, while 40% of the farms in SUDP system used AI service only. The average herd size per farm was 6.5 (range 2–24) and 9.9 (range 3–32) for the SUDP and MCLP systems, respectively, while the average number of cows per farm was 3.1 (range 2–10) and 3.2 (range 2–8), respectively. The overall mean (range) BCS was 3.8 (1–8), which was significantly influenced by district (location), production system, and proportion of exotic genes. Cows in the SUDP system had higher BCSs ($P < 0.001$) than did those in the MCLP system, while cows with a higher proportion of exotic genes (75–87.5%) had higher ($P < 0.05$) BCSs than did the others. In the five districts, cows in Wuchale-Jida had the lowest mean BCS (3.4).

## Reproductive and lactation performance

The overall geometric means for the calving-to-conception interval (CCI) and duration after last calving (DALC) were 187 (n = 382) and 201 (n = 422) for pregnant and non-pregnant cows, respectively (**Paper II**). District (location) and suckling status significantly influenced these two intervals. The two intervals were significantly longer in those cows that suckled their calves than in cows that did not. In the five districts, cows in Wuchale-Jida had the longest CCI and DALC, while cows in Sululta-Mulo had the shortest. The overall means for calving interval (CI), CCI, and first observed oestrus after calving were 516 (n = 47), 253 (n = 66) and 141 (n = 55) days, respectively (**Paper III**); suckling considerably prolonged these three intervals. The overall average numbers of services per conception were 1.6 (n = 382) and 1.7 (n = 69) in the cross-sectional (**Paper II**) and longitudinal (**Paper III**) studies, respectively.

Among the forty-three cows whose milk was analysed for progesterone (**Paper III**), the first onset of luteal activity (OLA) was observed in 25 (58.1%) of them during the follow-up period of a maximum of 90 days postpartum. The mean OLA after calving was 52 days (n = 25), while 67.4% had a delayed (>55 days) return to cyclicity. Based on the survival analysis method, suckling and parity number significantly influenced the OLA in the follow-up period, when those cows that suckled their calves and those that had parity number greater than three had a lesser chance and/or delayed resumption of ovarian cyclicity during the 90-day observation period compared to their respective counterparts.

Based on the longitudinal observation (**Paper III**), the mean lactation length was 54.4 weeks (n = 15, range 44–69 weeks) and the overall estimated daily mean milk yield for the first 43 lactation weeks was 11.7 L/day. The daily milk yield increased gradually and peaked at 13.8 L/day approximately 11 weeks postpartum, after which it declined gradually and steadily. Suckling status, season of calving and parity number significantly influenced the estimated average daily milk yield. The estimated daily milk yield was lower for those cows suckled their calves and those cows having a parity number greater than three, while the effect of calving season was variable in the two lactation length categories considered.

Based on the cross-sectional study (**Paper II**), the overall prevalence rates of abortion, pre-weaning calf mortality, dystocia, retained foetal membrane and vulval discharge/uterine infection were 1.4% (n = 940), 17.4% (n = 927), 1.3% (n = 940), 5.4% (n = 940) and 2.8% (n = 940), respectively. The incidence rates of abortion, pre-weaning calf mortality, dystocia, retained foetal membrane, uterine infection, and clinical mastitis observed in the longitudinal observation (**Paper III**) were 7.3% (n = 69), 10.9% (n = 64), 3.1% (n = 64), 7.8% (n = 64), 3.1% (n = 64), and 6.3% (n = 64), respectively.

## Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate, and progesterone (Paper IV)

The serum concentrations of PAG in Borana (n = 6) and crossbred (n = 8) cattle at 4 weeks post service were 1.5–5.5 ng/mL and 2.1–4.7 ng/mL, respectively. The mean PAG concentrations increased progressively from the $4^{th}$ to the $33^{rd}$ week of gestation in the ranges of 3.3–173 ng/mL for Borana and 4.2–240 ng/mL for crossbred cattle. The increase in PAG levels was drastic around parturition in both breeds, the mean (±SEM) concentration peaking at 1880±238 ng/mL and 1337±162 ng/mL in Borana (n = 7) and crossbred (n = 6) cattle, respectively) around parturition (on the day of calving or a day before or after calving). After parturition, the PAG concentrations decreased gradually and steadily to 5.7 ng/mL in Borana (n = 7) and 3.9 ng/mL in crossbred (n = 6) cattle 10 weeks postpartum.

The serum E1-S concentrations in Borana (n = 8) and crossbred (n = 9) cattle at the $17^{th}$ week of pregnancy were 0.3–2.6 and 0.9–5.7 ng/mL, respectively. The mean E1-S concentrations increased progressively from the $17^{th}$ to the $33^{rd}$ week of gestation, in the range 1.1–4.6 ng/mL for Borana and 2.7–10.8 ng/mL for crossbred cows. Breed, parity status, dam body weight, and foetal sex had a significant effect on serum concentrations of PAG and E1-S; foetal birth weight had a significant effect only on PAG. Crossbred cows, nulliparous (i.e., heifers), dams of lower body weight, and dams carrying female foetuses had higher serum PAG and E1-S concentrations than did their respective counterparts. Moreover, dams that carried foetuses of lower birth weight had higher PAG levels than did their counterparts.

The P4 concentrations at the $4^{th}$ week of pregnancy were 3.2–5.1 and 1.7–8.9 ng/mL in Borana (n = 6) and crossbred (n = 8) cattle, respectively. The serum P4 concentrations remained elevated throughout the gestation period. The serum P4 profiles varied considerably between the two breeds, the crossbred having significantly higher ($P < 0.05$) P4 concentrations than did Borana cattle in early gestation (weeks 4–13), while the Borana cattle had higher ($P < 0.01$) P4 levels than did crossbred ones in the later stages of pregnancy (week 17 and afterwards). A significant positive association was observed between serum PAG and E1-S concentrations (Pearson correlation coefficient of 0.45, $P < 0.001$).

# General discussion

This thesis describes the reproductive and lactation performance of smallholder dairy cattle with particular emphasis on pregnancy diagnosing and monitoring.

Although livestock play an important role in human society, livestock production is constrained by poor management and inadequate research, extension and veterinary support, as well as by ineffective communication between farmers and development agents in most developing countries, including Ethiopia. Despite the various challenges that smallholder livestock production faces, this sub-sector contributes significantly to the resource-poor family by providing food and draft power, and by generating income at both the household and national levels.

On the other hand, owning to Ethiopia's ever-increasing human population coupled with an increasing demand for milk and milk by-products, the development and expansion potential of the dairy sector in Ethiopia is great. Accordingly, the contribution of the dairy sector, especially the smallholder system, to poverty alleviation and sustainable food production in Ethiopia is thought to be quite high; the sector therefore deserves much attention, as increased knowledge and resource would allow optimal utilization of this potential for developing income and employment opportunities. In building our knowledge of several related aspects of this sector, this thesis contributes towards improving the performance of dairy cattle, in particular the smallholder dairy cattle in Ethiopia.

## Clinical features and profiles of pregnancy-associated glycoprotein, prostaglandin $F_{2\alpha}$ metabolite, and progesterone in association with abortion

Owing to the unpredictable occurrence of embryonic or foetal losses in a herd and current increasing interest in monitoring pregnancies in bovine, foetal death was induced in heifers using cloprostenol. Accordingly, the clinical features and plasma profiles of PAG, PG metabolite, and P4 were then monitored under controlled conditions.

It has been demonstrated that the clinical features and PG metabolite profiles in induced abortions between two stages of pregnancy, i.e., pregnancies below 75 days and between 100 and 150 days, were different (Lindell, Kindahl & Edqvist, 1980/1981). These authors reported that in the former group, aborted foetuses were delivered with intact foetal membranes with little or no bleeding and the heifers displayed standing oestrus immediately after abortion, whereas the latter group had blood-tinged vaginal discharge and retained foetal membranes. Our current observations (**Paper I**) confirmed the previous findings of Lindell, Kindahl & Edqvist (1980/1981), in that two of the heifers (at days 63 and 77 of gestation) had mucous vaginal discharge, expelled foetus with intact foetal membrane, and showed standing oestrus within two days of abortion. This finding has practical importance and confirms the suggestion of Lindell, Kindahl & Edqvist (1980/1981) that abortion could be induced as late as day 80 of pregnancy for practical reasons with little or no harm to the subsequent reproductive performance at least in heifers. The prompt resumption of cyclicity in this group may be partly because such abortions are associated with little or no uterine trauma due to the loose attachment of the foetal membrane to the uterus. This view is further supported by the return of PG metabolite release to the basal level immediately after abortion in the early pregnant group unlike those heifers above 100 days of pregnancy, as was observed by Lindell, Kindahl & Edqvist (1980/1981) and in the current study.

Detecting PSPB or bPAG1/PAG above the threshold levels in the maternal blood of cows or heifers is a good indicator of the presence of a live embryo or foetus with the except in the postpartum period or for a few days after embryonic/foetal death (Semambo *et al*., 1992; Szenci *et al*., 1998a). Current observations (**Paper I**) indicated that plasma levels of bPAG1 started to decline gradually within 48 hours of cloprostenol treatment (after expulsion of the foetuses) in three of the heifers and even later in the fourth heifer. However, the plasma level of bPAG1 did not fall immediately after death of the foetus, but it changed only slightly until the foetus was expelled. Such minor changes in the PAG levels may be due to the effect of uterine contraction caused by the pulsatile release of the endogenous $PGF_{2\alpha}$ (measured as the metabolite) and by the continuity of the placental release of bPAG1 for a brief time even after foetal death. Moreover, this experiment demonstrated that there was a tendency to variation in the rate of decline of plasma bPAG1 concentration among the heifers that had at different stages of gestation, which might be attributed to the increase in the plasma concentration and half-life of the glycoprotein with increasing stage of pregnancy. The gradual decline of PSPB/PAG concentrations after induced embryonic/foetal mortality by experimental *Arcanobacterium pyogenes* infection or cloprostenol injection has been found in previous reports (Semambo *et al*., 1992; Szenci *et al*., 2003).

The plasma PG metabolite levels increased immediately after cloprostenol treatment, and continued to increase with pulsatile release until expulsion of the foetus. However, in heifer no. 4 (which was at the highest gestation stage of the four heifers), the pulsatile release of PG metabolite continued up to five days post cloprostenol injection even after expulsion of the foetus, though the highest concentration of PG metabolite was only 623 pmol/L. This plasma PG metabolite concentration is low compared to that previously reported by Lindell and co-workers (1980/1981), who reported the massive release of PG metabolite up to 2500 pmol/L in heifers at the same pregnancy stage as was heifer no. 4 at the time of abortion induced by cloprostenol. This difference in the level of PG metabolite could be partly attributed to the effect of retained foetal membranes in case of the latter heifers displaying higher levels of PG metabolite. The observed sharp decline of plasma levels of progesterone in all heifers within 24 hours after the cloprostenol injection is in agreement with previous reports that indicated the luteolytic effect of cloprostenol in cyclic non-pregnant or pregnant cows/heifers (Lamond *et al*., 1973; Kindahl *et al*., 1976; Lindell, Kindahl & Edqvist, 1980/1981; Bekana, Odensvik & Kindahl, 1996). In this study (**Paper I**), the disruption of the foeto–endometrial connection because of uterine contraction caused by the pulsatile release of the endogenous PG metabolite could possibly have caused the foetal deaths within 24 to 48 hours of cloprostenol injection.

A sharp drop in progesterone and a gradual decline in PAG concentrations were observed in a Borana cow that experienced spontaneous abortion after approximately 10 weeks of gestation (**Paper IV**). A similar pattern of PAG and P4 profiles has been reported by various authors after experimentally induced embryo/foetal deaths (Semambo *et al*., 1992; Szenci *et al*., 2003; **Paper I**). Owing to the unprecedented occurrence of spontaneous abortion, the current finding, particularly of the serum profiles of PAG, is valuable. It provides a good example of the real situation in which some animals might abort and confirms those previous results obtained experimentally using induced abortions.

## Reproductive and lactation performance

In this thesis (**Papers II** and **III**), the reproductive performance of smallholder dairy cows in the Selalle area was evaluated: by estimating the intervals from calving-to-conception, to the next calving, to first observed oestrus, and to the first onset of luteal activity; by estimating the duration after last calving (for non-pregnant cows); and by measuring the average number of services per conception. The cross-sectional study (**Paper II**), which involved 300 smallholder dairy farms in five districts, found that the mean estimated CCI was 187 days. The extended mean CCI estimated in the cross-sectional study was further confirmed by the longitudinal observation (**Paper III**) that the intervals from calving-to-conception, to the next calving, and to the first observed oestrus were also extended. This interval (CCI) is the main component that determines length of calving interval, which in turn is the parameter of interest for evaluating the reproductive efficiency of dairy cattle.

The current overall mean estimates of CI, CCI, and first observed oestrus fall within the ranges reported for crossbred and local Zebu cattle in different management systems in Ethiopia (Swensson *et al*., 1981; Albero, 1983; Shiferaw *et al*., 2003) and for crossbred cattle in the tropics (Obese *et al*., 1999; Masama *et al*., 2003; Swai *et al*., 2005). Although the present estimate is within the range of previous reports, it is unfavourable and extended compared with the optimum CCI (80–85 days) recommended to achieve the commonly accepted one-year calving interval (Roberts, 1986; Peters and Ball, 1995; Arbel *et al*., 2001). The findings of this thesis (**Papers II** and **III**) together with previous similar reports from Ethiopia suggest that the reproductive efficiency of smallholder dairy cattle is unsatisfactory and hence deserves attention.

The present work (**Paper II**), found the CCI to be more extended ($P < 0.05$) in the MCLP than in the SUDP system and higher ($P < 0.01$) in suckling than in non-suckling cows. Shiferaw *et al*. (2003) reported a similar finding from other parts of the country, where CCI was longer in the MCLP than in the SUDP system. The prolonged CCI observed in both production systems (**Paper II**) may be partly attributed to inadequate nutrition, poor health services, and other management-related problems. On the other hand, the nutritional deficiencies, particularly in the long dry season of the year, and heavy parasitic infestations of cattle in the MCLP system, in which extensive grazing is practiced, might have contributed to the differences in the estimates of CCI between the two production systems.

Moreover, the three intervals (CI, CCI, and first observed oestrus) considered in the longitudinal study (**Paper III**) were significantly longer in those cows that suckled their calves than in those that did not. Similarly, the results of survival analysis (**Paper III**) indicated that cows that suckled their calves had a lesser chance and/or a delayed resumption of ovarian activity in the 90-day follow-up period after calving compared to their respective counterparts, further confirming the important effect of suckling on the reproductive performance of smallholder dairy cows. These findings are supported by the fact that suckling could impair the release of luteinising hormone and subsequently interfere with the resumption of ovarian activity (Carruthers and Hafs, 1980; Peters, 1984; Spicer and Echternkamp, 1986). In addition, the milk progesterone analysis (**Paper III**) indicated that major proportion (67.4%) of the cows followed experienced a delayed (>55 days) first onset of luteal activity. Similarly, Lyimo *et al.* (2004) from Tanzania reported that 55% of cows followed experienced a delayed (>60 days) postpartum resumption of ovarian activity. Moreover, a postpartum anoestrous interval of 98 days was reported in smallholder crossbred cows (Kassa, 1990).

The intervals from calving-to-conception and to first observed oestrus were considerably prolonged (**Papers II** and **III**) compared to the postpartum anoestrous interval found in the current (52 days) and previous studies. In contrast, the average number of services per conception found in both the cross-sectional study and longitudinal observations seems satisfactory. Accordingly, one could speculate from these findings that it is not only that the delayed postpartum anoestrus that plays a role in the extended intervals observed in the smallholder cows in the study area; possibly silent oestrus, poor oestrous detection, or other factors, such as farmer decision's or ignorance, could also attribute to such extended intervals. This speculation is further supported by a previous report that oestrus was missed once or several times in 34.7% of smallholder crossbred cows in Tanzania (Lyimo *et al.*, 2004). Generally, other factors such as inadequate nutrition, poor housing systems, inefficient heat detection, and incomplete or total lack of recording systems might have also contributed considerably to the observed prolonged intervals.

The observed lack of a clear pattern with regard to the effect of calving season (**Paper III**) may be partly caused by the inconsistency and great variation in type and amount of supplemental feed given to smallholder cows in the study area (**Paper II**), which depending on various factors. On the other hand, the current work (**Paper III**) demonstrated that older cows (parity number greater than 3) had a reduced chance and/or delayed onset of luteal activity than younger ones, though production (milk yield) was found to have no effect ($P > 0.05$) on the resumption of luteal activity. However, it is known that in high-milk-yielding cows negative energy balance can prolong postpartum anoestrus. Therefore, the current finding may be attributed to the reduced occurrence or mitigated severity of negative energy balance in the low- or medium-milk-yielding crossbred cows owned by smallholder farms; as well the effect of production could possibly be masked by the stronger effect of parity observed in the current study as the number of animals included was small.

In view of various previous reports, the current findings about the rates of reproductive disorders (**Papers II** and **III**) in smallholder dairy farms are not serious. Prevalence rates of 14.7% retained foetal membrane and 15.5% uterine infection were reported in other parts of Ethiopia (Shiferaw *et al*., 2005). In contrast, Swai *et al*. (2005) reported much higher prevalence rates of dystocia (58%) and retained foetal membrane (17%) in smallholder crossbred dairy cows in Tanzania. Similarly, in dairy herds in the UK, 9%, 3.6%, 15–22%, and 1.5% annual incidences of dystocia, retained foetal membrane, vulval discharge/endometritis, and abortion, respectively, have been reported (Esslemont and Peeler, 1993; Esslemont and Kossaibati, 1996). Several factors, such as herd size, production level, incidence of various diseases, genetics, housing, and management could influence the rates of occurrence of the various reproductive disorders, which might have contributed to the observed variations among the different reports.

The longitudinal observation (**Paper III**) estimated a mean lactation length of 54.4 weeks, which fell within various previously reported ranges for smallholder crossbred cows in the tropics (Teodoro and Madalena, 2003; Masama *et al*., 2003; Msangi, Bryant & Thorne, 2005). Unlike most modern dairy farms, where a lactation length of 305 days is commonly accepted as standard, the current estimate could be considered as extended. However, such extended lactation length has practical significance to the smallholder dairy farmers, as it allows compensation for the usually extended calving intervals, given that crossbred cows kept in smallholder dairying systems frequently have flat, persistent lactation ‘curves’ (Tanner, McCarthy, & Omore, 1998). The profitability of short versus extended lactation length depends on various factors, including the persistency of the lactation. For example, Borman, Macmillan & Fahey (2004) suggested that extended lactations are a suitable option for some dairy enterprises, and the suitability depends particularly on cow milk potential, ability to economically grow pasture or feed supplements, management expertise, environmental constraints, herd size, and labour availability.

The estimated mean daily milk yield (11.7 L/day) for the first 43 lactation weeks (**Paper III**) is higher than some other reports from smallholder crossbred dairy cattle in tropical countries, which range from 6.5 to 9.8 L/day (Msanga and Bryant, 2003; Teodoro and Madalena, 2003; Muraguri, Mcleod & Taylor, 2004; Msangi, Bryant & Thorne, 2005). Moreover, the maximum estimated daily milk yield record (27 L/day) is higher than that of Muraguri, Mcleod & Taylor (2004), who reported production of 19.5 kg/day in smallholder dairy cattle in Kenya. In view of the other reports mentioned above, the current estimated mean daily milk yield and relatively high maximum daily milk yield from crossbred cows managed at smallholder production level are encouraging. In addition, the gradual and steady decline of average daily milk yield by 1.5–7.0% per month after the peak made the lactation curve somewhat flat, compared to the usual lactation curve of high-yielding cows that declines by 8-10% per month after the peak (Esslemont, 2003).

On the other hand, the current study found that peak milk yield was reached later than has been found in most studies of high-yielding cows. The prevailing inconsistency in the type and amount of supplemental feed provided to smallholder dairy cows (**Paper II**), as well as other management factors, might have partly contributed to the observed delay in peak milk yield after calving.

The current work (**Paper III**) demonstrated that suckling status, season of calving and parity number significantly influenced the estimated weekly average daily milk yield, in contrast to previous reports that found suckling had no or little effect on milk yield (Msanga and Bryant, 2004) or mean total milk off-takes up to weaning (Little, Anderson & Durkin, 1991). However, the current work did not consider the milk consumed by the suckling calf, which was estimated to be 2.4 to 2.8 L/day by other researchers (Msanga and Bryant, 2003; Msangi, Bryant & Thorne, 2005). On the other hand, Msangi, Bryant & Thorne (2005) demonstrated that calving year, calf-rearing method and BCS significantly influenced the daily milk yield of the smallholder crossbred dairy cows in Tanzania, where restricted suckling increased the milk yield considerably more than did the bucket-fed method of calf rearing. Thus, the difference observed in the current work between suckling and non-suckling cows might have not been observed if the milk sucked by calf had been estimated and subsequently included in the analysis. The observed effect of season of calving could be partly attributed to the prevailing seasonal fluctuations in feed availability and supplementation of smallholder dairy cattle.

## Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate, and progesterone (Paper IV)

This thesis established the serum profiles of PAG, E1-S, and P4 throughout gestation and early postpartum (for PAG only) in Ethiopian Borana and their crossbred cattle.

This work demonstrated that all blood samples collected and analysed from Ethiopian Borana and their crossbred pregnant cattle had well detectable levels of PAG by the 4$^{th}$ and E1-S by the 17$^{th}$ week post conception. In addition, the PAG values obtained from all blood samples analysed were higher than the previously established cut-off value of 0.8 ng/mL (Skinner *et al*., 1996) or 0.5 ng/mL (Szenci *et al*., 1998b) needed for a positive pregnancy diagnosis. The highest PAG value observed in the present study by the 4$^{th}$ week post service is comparable to the value (5.3 ng/mL in Holstein-Friesian cows at day 26 or 27 post service) found by Szenci *et al*. (2000). As PAG and E1-S are specific to pregnancy, they are reliable indicators of viable pregnancy (Semambo *et al*., 1992; Szenci *et al*., 1998a for PSPB or PAG and Hamon *et al*., 1981; Takahashi *et al*., 1997; Hirako, Takahashi & Domeki, 2002 for E1-S) and could be used for pregnancy diagnosis and monitoring foetal wellbeing. Moreover, PAG is thought to be a promising early pregnancy diagnostic tool, provided its often-cited drawback of a long half-life and carry-over antigen in the early postpartum period is solved. Therefore, the serum PAG and E1-S levels established in the current work could be useful as a reference points in the future, at least for research purposes in Ethiopia.

The current observation indicated that both serum PAG and E1-S concentrations increased progressively starting from the first sampling (in the 4$^{th}$ and 17$^{th}$ weeks of gestation for PAG and E1-S, respectively) until the 33$^{rd}$ week of gestation in both Borana and their crosses. A similar trend in plasma PAG profiles was reported by Sousa *et al.* (2003) in zebu cattle, while the progressive increase in E1-S concentrations is in agreement with the reports of Dobson *et al.* (1993) and Takahashi *et al.* (1997). However, there were some variations between the present findings and previous ones with regard to peak PAG concentrations occurring around parturition. The peak values (1880 and 1367 ng/mL in Borana and crossbred cattle, respectively) found at calving in the present research are lower than those of Zoli *et al.* (1992) and Kornmatitsuk *et al.* (2002) who found peak PAG values of 2462 ng/mL in Holstein-Friesian pregnant heifers and 3100 ng/mL in Swedish pregnant heifers, respectively; however, the current values are higher than those (1096 ng/mL) reported for zebu cows by Sousa *et al.* (2003). Owing to the drastic changes in PAG levels occurring around parturition, the observed differences among the different reports could partly be attributed to differences in the time and date of blood sampling and possibly to breed differences.

On the other hand, in the prepartum period, the E1-S profile increased dramatically in both breeds but started to decline at parturition week in Borana cattle, while remaining elevated in crossbred cattle. Likewise, Takahashi *et al.* (1997) reported variations of prepartum profiles, in which singleton cows exhibited undulation in E1-S profile at prepartum unlike twin-bearing cows, which displayed a markedly elevated profile until the day of parturition. The present work also demonstrated that the PAG levels in both breeds declined gradually after delivery and that the levels remained high at least until 10 weeks postpartum, in agreement with the various previous reports regarding both zebu and Holstein cows (Zoli *et al.*, 1992; Sousa *et al.*, 2003). The notably high (above the cut-off value) PAG concentrations detected up to 10 weeks postpartum in the present work further confirms the limitations of the currently available PAG assay test for use as a pregnancy diagnosis tool in the early postpartum period; further research to improve the technique is thus required.

This thesis (**Paper IV**) demonstrated that breed, dam weight, foetal sex, and parity status (parous versus nulliparous) had considerable effect on the serum PAG and E1-S concentrations during gestation. Likewise, Zoli *et al.* (1992) reported that breed and foetal sex significantly influenced the peripheral blood levels of PAG in the peripartum period. In contrast, Kiracofe *et al.* (1993) found that neither cow weight, body condition score, sex of calf, nor calf birth weight affected the concentrations of pregnancy-specific protein B (PSPB) in beef cows. As well, Robertson and King (1979) demonstrated that the E1-S concentration in plasma and foetal fluids varied considerably during gestation in the cow. Moreover, E1-S concentration in plasma or serum was reported to be higher in twin than in singleton pregnancies in the cow (Worstfold, Williams G. & Williams D., 1989; Dobson *et al.*, 1993; Takahashi *et al.*, 1997).

The present work estimated considerably higher concentrations of PAG and E1-S in dams of lower body weight (less than 275 kg) than in those of higher body weight (275 kg or more) during gestation. Interestingly, a higher PAG concentration in the last two trimesters of pregnancy in a zebu cow that had a much lower BCS than did the other studied cows was reported by Sousa *et al*. (2003). Thus, this finding could be interesting and it opens door for further research to investigate the possibility of influencing the secretion of PAG and E1-S by the binucleate cells of the placenta by manipulating the nutritional status (BCS) of pregnant cows/heifers. Moreover, this thesis (**Paper IV**) also demonstrated the existence of a positive correlation between PAG and E1-S levels, an observation comparable with a previous report indicating a positive association between PSPB and E1-S levels (Dobson *et al*., 1993). The observed positive association between PAG and E1-S levels might be because of their common placental origin.

Although the milk P4 test is employed for routine pregnancy diagnosis in lactating cows, determining the peripheral blood level of P4 has practical importance particularly in heifers, for pregnancy diagnosis and/or monitoring embryo/foetal viability in Ethiopia, at least for research purposes. Accordingly, this thesis (**Paper IV**) demonstrated that the serum P4 concentrations starting from the first blood sampling (in the $4^{th}$ week of gestation) remained elevated throughout gestation in both breeds, which is consistent with data in Brahman (Shelton and Summers 1983), zebu (Mukasa-Mugerwa *et al*., 1989), and taurine (Robertson, 1972) cattle. Moreover, the present research found that P4 levels started to decline approximately three weeks prepartum, a finding in agreement with other reports (Smith *et al*., 1973; Mukasa-Mugerwa *et al.*, 1989). Mukasa-Mugerwa *et al*. (1989) reported that P4 levels varied with the week of pregnancy in zebu cattle, which is comparable to our finding at the later stages of pregnancy.

# General conclusions and recommendations

- After early cloprostenol-induced foetal death (at between 60 and 120 days of pregnancy) the plasma concentrations of bPAG1 decreased gradually and the rate of decrease displayed a tendency to vary with the stages of pregnancy, a matter that requires further confirmation.
- The current finding further supported the suggestion of Lindell, Kindahl & Edqvist (1980/1981) that abortion could be successfully induced up to 80 days of pregnancy for practical reasons, at least in heifers with little or no harm to subsequent reproductive performance.
- The prevalence of pre-weaning calf mortality was notably high in the smallholder dairy farms studied and deserves serious attention.
- The smallholder dairy cows in the study area had prolonged intervals from calving-to-conception, to the next calving and to the first observed oestrus after calving, and thus their reproductive performance was unsatisfactory.
- Delayed first postpartum oestrus and possibly silent oestrus or inadequate oestrous detection, or other factors (which require further investigation) might have contributed to the extended intervals.
- The current work estimated the lactation performance of smallholder crossbred cows based on the average of two days of milk yield records per lactation week, and involved a small number of cows with complete lactations and did not estimate the milk sucked by the calf. Therefore, further detailed investigation is suggested to examine the effect of such factors as suckling regimes (including estimating the milk sucked by the calf), body condition scores of cows at calving and types and amounts of feeds supplied on the reproductive and lactation performance of smallholder crossbred dairy cows for the subsequent implementation of appropriate intervention measures.
- The serum concentrations of PAG and P4 at the $4^{th}$ and E1-S around the $17^{th}$ week of pregnancy were well above the required levels for pregnancy testing in both Borana and crossbred (Borana × Holstein-Friesian) cattle.
- The observed serum profiles of PAG, P4, and E1-S throughout gestation and for ten weeks postpartum (for PAG only) are comparable to the previous reports from other cattle breeds.
- The serum PAG and E1-S profiles were considerably influenced by factors such as breed, weight and parity status of the dam, and foetal sex; however, foetal birth weight influenced only PAG.

## Scope of future research

- The observed high rate of pre-weaning calf mortality warrants attention, so further investigation aimed at exploring the major causes of pre-weaning calf mortality is vital.
- Assessing the comparative effects of different regimes of restricted suckling on reproductive and lactation performance.
- Evaluating the nutritional values and economic feasibility of various locally available animal feed resources for feeding smallholder dairy cows.
- Further research to improve the skill of AI technicians and animal health professionals (involved in the dairy sector) at early pregnancy diagnosis and fertility evaluation.
- Looking for a simple and feasible early pregnancy diagnostic tool that could be applied under smallholder farm conditions.
- Further work on improving smallholder farmers' awareness of the breeding and management of crossbred dairy cattle (using a participatory approach) is imperative.

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