ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

STUDY ON FACTORS AFFECTING THE SUCCESS OF ARTIFICIAL INSEMINATION PROGRAM IN CATTLE, SILTIE ZONE

BY

HAMID JEMAL

JUNE 2012

DEBRE ZEIT, ETHIOPIA

STUDY ON FACTORS AFFECTING THE SUCCESS OF ARTIFICIAL INSEMINATION PROGRAM IN CATTLE, SILTIE ZONE

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HAMID JEMAL

A thesis presented to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Veterinary Obstetrics and Gynaecology

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ABBREVIATIONS

AI	Artificial Insemination
ART	Assisted Reproductive Technology
CI	Calving Interval
FAO	Food and Agriculture Organization of the UN
GnRH	Gonadotropin Releasing Hormone
ILCA	International Livestock Center for Africa
ILRI	International Livestock Research Institute
IOI	Insemination-Ovulation Interval
LH	Luteinizing Hormone
masl	meters above sea level
MoA	Ministry of Agriculture
NAIC	National Artificial Insemination Center
NSPC	Number of Service per Conception
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
pp	post partum
RFM	Retained Fetal Membrane
SCR	Service per Conception Rate

ABSTRACT

Efficiency of artificial insemination service might be lower due to various factors including failure of estrus detection, poor management, inadequate nutrition and poor quality of semen. Failure of estrus detection and improper timing of insemination has been the single most known factor in dairy animals. In this study, the performance of the conventional method of AI delivery was evaluated to compare with improved estrus detection method through artificial estrus induction. Pregnancy rate was compared between dairy animals inseminated by the conventional way and those inseminated after estrus induction using synthetic prostaglandin. Pregnancy rate for the whole of the study group using AI was 42.9%. There was also a significant breed difference in pregnancy rate ($p<0.05$, 34.4%; n=203 local breeds, and 66.6%; n=72 in cross breeds) among the study animals. The pregnancy rate was 48.1% (n=77) for induced animals while it was 28.2% (n=85) for those that were inseminated without estrus induction. There was a significant difference ($p<0.05$) in pregnancy rate between induced and non induced animals. The mean (±SE) length of induction to first estrus sign and estrus to AI were 57.49±1.9 hrs and 8.62±0.15 hrs (range 6-12.3 hrs), respectively. The overall mean (±SE) service per conception in conventional insemination service was 2.34±0.05. Bellowing, standing to be mounted and vaginal mucus discharges were found to be the most common estrus manifestations observed by dairy owners as a reason for AI. Animals inseminated after vaginal mucus discharge had a significantly higher rate of pregnancy compared to other estrus signs. There was also a significant difference ($p<0.05$) in pregnancy rate among animals inseminated at different times with the highest record being for animals brought on-time (6-12 hrs of first estrus manifestation). In conclusion, estrus manifestation and body conditions were the two most important factors associated with pregnancy rate. The use of artificial induction of estrus has improved the success of AI though dramatic changes were to be obtained only after a concomitant improvement of the body condition of the cows.

Keywords: AI, Cows, Estrus detection, Estrus induction, Pregnancy rate

1. INTRODUCTION

Artificial insemination (AI) is the manual placement of semen in the reproductive tract of the female by a method other than natural mating and it is among the group of technologies commonly known as ―assisted reproduction technologies" (ART), whereby offspring are generated by facilitating the meeting of male and female gametes (Morrell, 2011). Since the early attempts by Italian physiologist Spallanzani, there has been considerable effort in the development of semen preservation and AI technology (Katherine and Maxwell, 2006). At present, fresh, chilled and frozen-thawed semen are used extensively for AI in animal breeding and production throughout the world (Ball and Peters, 2004). Selective breeding has been considered as one of the highly effective and sustainable approaches for increasing animal productivity in the long-term. In this regard, AI allows a single animal to have multiple progeny, reducing the number of parent animals required and allowing for significant increases in the intensity of selection, and proportional increases in genetic improvement (Webb, 1992; Arthur, 2001; Morrell, 2011). Reproductive efficiency has been reported to be the product of successful estrus detection and conception rates (Bekana *et al*., 2005). Improvement in livestock resources can be achieved through the implementation of an efficient and reliable AI service, concomitantly with proper feeding, health care and management of livestock. Hence crossbred cattle, the progeny of zebu and Holstein Frisian/jersey cattle are mainly used for milk production in this country which is concentrated in dairy farms around the major cities (Heinonen, 1989; Bekele *et al*., 1991; Belihu, 2002; Hassen *et al*., 2007).

Achieving increased milk and meat production through genetic improvement of indigenous cattle have been the primary goals of the livestock development plan of Ethiopia (Heinonen, 1989). Paradoxically, though AI program continued for several decades, the genetic improvement achieved to date is very unsatisfactory due to several factors. Currently only 151,344 (0.35%) of the total cattle population are hybrids and 19,263 (0.04%) heads are exotic breeds (Shiferaw *et al*., 2003; Demeke, 2010). A number of factors such as poor heat detection, inefficient AI delivery system, and poor semen quality are known major causes of poor success of AI programs

(Pursley *et al.*, 1997; Chebel *et al.*, 2004; Overton and Sischo, 2005; Salverson and Perry, 2005). The percentage of pregnancies resulting from AI is the product of cows detected in heat and inseminated, fertility level of the herd, semen fertility level and inseminator efficiency (Risco, 2000; Heersche, 2002). Collectively, errors in efficiency and accuracy of heat detection result in high semen cost and an increase in the interval from calving to conception, reducing cow production and net returns.

Despite the wide application and success of AI throughout the developed world, the success rate in African and other developing countries is still low owing to a number of technical, system related, financial and managerial problems (Tegene *et al.*, 1995; Belihu, 2002). According to the report of Chupin and Schuh (1992), AI coverage, semen production per year and AI applications per inseminator in Africa remains low. The same report indicated that the average AI application per year per country in Africa is 30,637 which is far below the values for Asian, Latin America and Near East countries. In addition, the pregnancy rate in AI scheme does not exceed 45% in Africa and the conception rate to first service was 48% in zebu cows kept at the Ministry of Agriculture Ranch in Ethiopia (Mukasa-Mugerwa *et al.*, 1991). Among the constraints are poor heat detection, communication and transport problems that hamper timely insemination, poor semen processing and storage technology affecting semen quality, and inefficiency of AI technicians (Belihu, 2002).

Circumstantial evidences indicate similar conditions prevail in most parts of Ethiopia regarding efficiency of AI programs. Particularly in Silte Zone, only 2,556 (0.48%) are cross breeds out of 525,178 cattle population present. Though recently two liquid nitrogen plants were established within the SNNPR to promote the AI program, the service has been constrained by different factors including poor heat detection that have not been objectively studied and addressed.

Therefore, the objectives of the current study were:

- To describe the facets of the AI program in Siltie Zone and determine the factors affecting its success
- To evaluate post AI pregnancy rate by enhancing estrus detection using Estrumate

- Compare efficiency of AI as measured by pregnancy rate through the conventional method employed by MoA and the one after induction of estrus

2. LITURATURE REVIEW

2.1. Artificial Insemination in Bovine

The use of AI as a tool to enhance production efficiency in cattle (Holm *et al*., 2008) and the successful use of artificial insemination (AI) as a means of animal breeding relies upon three major premises: firstly, that spermatozoa can survive outside the body; secondly, that they can be reintroduced into the female genital tract in a way that results in an acceptable conception rate; and thirdly, that the fertile period of the female can be identified (Arthur, 2001; Holm *et al*., 2008; Manafi, 2011). Artificial insemination offers several potential advantages over natural service, of these, the reason most commonly advocated is as a means of genetic improvement and others include cost effectiveness, disease control, safety breeding, flexibility, and fertility management (Ball and Peters, 2004). Cows ovulate at about 12 hours after the end of the estrus period. The ideal time for insemination is therefore 6–24 hours prior to ovulation (Arthur, 2001; Ball and Peters, 2004).

Services needed per conception relates directly to the conception rate (CR %). At conception rates of 70, 60 and 50%, respectively, 2.7, 6.4 and 12.5% of healthy cows need 4 services to conceive (Table 2). Bearing that in mind however, for practical purposes, a "repeat breeder" can be defined as a female that needs ≥4 services to conceive. If >12.5% of cows need 4 or more services, the herd has a moderate to severe fertility problem (Rogers, 2001). According to his report the overall features of bovine fertility describing reproductive indices and herd fertility in different category is presented in the following table (1).

Table 1: Indices to assess the severity or otherwise of a reported problem of "herd infertility"

Assessment index	"Normal" background (very good to good)	Slight problem	Moderate problem	Severe problem
% pregnant to a given service	>65.0-60.1	50.1-60.0	45.0-50.0	<45.0
Services/Conception of all served cows	<1.54-1.66	1.99-1.67	2.22-2.00	>2.22
Calving to Service Interval (days)	<60.0-65.0	70.0-65.1	85.0-70.1	>85.0
% Non-Detected estrus	<10.0-14.9	19.9-15.0	40.0-20.0	>40.0
% Heat Detection Rate	>90.0-85.1	80.1-85.0	60.0-80.0	<60.0
% 18-24 day return intervals	>65.0-62.5	60.1-62.4	50.0-60.0	<50.0
% Needing 3 services	<12.3-15.9	24.9-16.0	30.2-25.0	>30.2
% Needing 4 services	<4.3-6.3	12.4- 6.4	16.6-12.5	>16.6

Source: Rogers, (2001)

Pregnancy rate to a given service is calculated separately for 1st, 2nd, 3rd, 4th services, or for all services as: 100 x numbers of dams pregnant (confirmed) to that service/ number of dams given that service. As a guide to herd fertility, the percent (%) pregnant to 1st service is most useful, as it may identify problems early on. Services per conception for all served dams were also defined as total services divided by the total number of dams pregnant.

Table 2: Conception rate and subsequent number of services

	% of healthy cows that need		
CR%	2 services	3 services	4 services
70	30	9.0	2.7
65	35	12.3	4.3
60	40	16.0	6.4
55	45	20.3	9.1
50	50	25.0	12.5
45	55	30.3	16.6

Early identification of both pregnant and non pregnant cows improves reproductive efficiency pregnancy rate in cattle by decreasing the between service interval. Many new and old technologies such as rectal palpation, ultrasonography, milk progesterone test and early pregnancy factor are available to identify pregnancy. The accuracy of pregnancy detection varies accordingly. Rectal palpation is the easiest and cheapest method that can be carried out from day 35 – 65 pregnancy. Its accuracy beyond day 60 reaches 95% (Broaddus and de Vries, 2005). In general, ultrasound diagnosis of pregnancy can be made as early as day 25 after insemination. An evaluation of this diagnostic method has demonstrated excellent sensitivity (detection of pregnancy when the animal truly gravid) greater than 95% at day 26 post AI (Filtean and Des Cõteaux, 1998). From diagnostic point of view it has greatest advantage for practitioners to obtain an excellent predictive value of negative pregnancy (in non gravids). The accuracy of pregnancy detection after day 30 reaches to 98-100% (Filtean and Des Cõteaux, 1998).

2.2. Factors Affecting Success of AI

The successful outcome of AI in cattle depends on a number of intrinsic and extrinsic factors. Understanding the impact of such factors on the probability of success of AI has primary importance to establish corrective measures (Haugan *et al*., 2005). Reproductive efficiency is poor in most cattle production systems, mainly cows fail to become pregnant due to various factors related to management, nutrition, reproductive diseases, semen quality and other factors, so that the extension service must ensure that farmers get adequate information on the input required to benefit from crossbreed dairy cows and from those of higher genetic merit (Mekonnen *et al*., 2010).

2.2.1. The role of the AI delivery system

A number of factors have made AI delivery system in Ethiopia inefficient. Among these factors are inefficient management, lack of integration of AI service with livestock health and feed packages, absence of appropriate collaboration among stakeholders, poor motivations and skills of inseminators, lack of readily available inputs such as liquid nitrogen, and absence of proper recording systems (Shiferaw *et al*., 2003). Some farmers have to move their cows for long distance in search of AI service. This is happening in many areas and the reason is AI technicians are unable to get transport facilities like motor bicycles, fuel, etc. AI is known to be a time dependent activity, in which during this long journey/waiting time, heat period is passed away before the service have been given (Lemma, 2010).

2.2.2. Factors related to heat detection and time of insemination

Knowledge of estrus behavior and the estrus to ovulation interval is essential for estimating the best time to artificially inseminate cattle though this have been not well characterized for zebu breeds (Pinheiro *et al.*,1998; Barros *et al*., 2006; Reolofs *et al*., 2010). According to various authors (Hafez, 1993; Arthur, 2001; Morrell, 2011), two phases of the estrous cycle have been documented: follicular and luteal phases. The follicular phase comprises proestrus and estrus, and behavioral estrus and ovulation occur during this phase. In European breeds AI is performed 12 hr after the beginning of estrus to allow for the presence of capacitated spermatozoa in the oviduct at the time of ovulation. In Bos taurus breeds estrus behavior lasts approximately 18hr and ovulation occurs 28 to 31 hrs after the onset of estrus. However in Bos indicus estrus is shorter lasting approximately 10-15 hrs and ovulation occurs around 25 hrs after the start of estrus (Pinheiro *et al.,* 1998; Arthur, 2001; Ball and Petters, 2004; Reolofs *et al*., 2010). Other studies also have been suggested that conception rate will not be reduced substantially if cows are inseminated with in 6hrs after initial observation of estrus (John, 2012). AI conducted at approximately 12 hrs after onset of standing heat potentially has lower fertilization rate with increasing embryo degeneration (Roelofs *et al*., 2010 and Dalton, 2011).

The duration of standing estrus has been reported by several authors to be shorter in zebu than in taurine cattle. Zebu cows have a shorter (10.7 - 11.6hr), less intense estrus which occurs later relative to the estrogen stimulus. As with taurine cattle, the duration of estrus and estrous cycle length tend to be shorter in zebu heifers than in cows (Galina *et al*., 1996). Zebu cattle have low fertility and this low fertility was reported to be due to poor heat detection, poor signs of estrus, and irregularity of the estrous cycle (Rekwot *et al*., 1999).

Studies have shown that up to 15% of the cattle presented for insemination are really not in heat (Chaundhari and Sabo, 2006) and on the other hand inaccurate heat detection is cost to the owners; in research it has been estimated between 5-25% of cows are not in heat when inseminated and a heat detection accuracy of 90% (2 of every 20 cows inseminated are not in heat) has been an acceptable goal (Chaundhari and Sabo, 2006). Compared with other factors accuracy of heat (estrus detection) is one of the major factors that determine the efficiency of an AI program. Heat detection in cows is carried out by experienced herdsmen/inseminators who are able to identify those animals with standing heat. Since the period is the shortest period between two successive estrus cycles a meticulous observation is required (Arthur, 2001; Iftikhar *et al*., 2009). As shown in (Table-3), animals in standing heat also manifest other behavioral symptoms like ‗soliciting' another cow to mount, standing to be mounted, chin resting, frequent urination, bellowing, raised tail, restlessness and standing to be mounted besides to different visible external changes like vulvular edema and absence of wrinkles on vulvular lips, vaginal hyperemia, wetness and mucus discharge (Ball and Petters, 2004). Almost 86% of females who ride other females (under the domain of the estrogen) are in estrus or proestrous (Arthur, 2001; Morrell, 2011). Roelofs *et al*. (2006) mentioned that expression of estrus can be influenced by many factors such as heritability, number of days at postpartum, lactation number, milk production, and health. Environmental factors like nutrition, season, housing, herd size, etc. also play a role in estrus manifestation.

Table 3: Timing of estrus detection in cattle

	Timeline for Heat Signs in Cattle		
	Coming into Heat	Standing Heat	Going out of Heat
	(6 hours)	(12 hours)	(14+ hours)
Heat Signs	Stands and bellows Smells other cows Head butts other cows Attempts to ride other cows but will not stand to be mounted Red, moist, slightly swollen vulva Clear mucous discharge from vulva	Stands to be mounted Rides other cows Bellows frequently Nervous and excitable	Attempts to ride other cows but will not stand to be mounted Smells other cows Clear mucous discharge from vulva

Adapted and modified from Jane *et al.* (2009)

According to Jane *et al.* (2009) standing heat can occur any time in a 24-hour period. However, the most likely time for a cow or heifer to show heat signs is at night but the season of the year can influence this, with more cows showing heat at night in hot weather and more showing heat during the day in cold weather. Hot weather, high production, crowded conditions, and high stress environments may reduce mounting activity. Observers must distinguish among cattle coming in to heat, in standing heat, and going out of heat. Females that are in standing heat, were in standing heat yesterday, or will be in standing heat tomorrow are the most likely herd mates to mount other cows or heifers in heat (Jane *et al.,* 2009).

The optimal time at which insemination should take place relative to ovulation (insemination–ovulation interval = IOI) depends mainly on the fertile lifespan of spermatozoa and on the viable lifespan of the oocyte in the female genital tract (Roelofs *et al*., 2006). For conception to occur, insemination must take place at the correct stage of the cow‘s estrus cycle since ova remains viable for about 12-18 hrs after ovulation (Rodriguez-Martinez, 2000; Cartmill *et al*., 2001).

Successful fertilization highly depends on the time interval from insemination to ovulation meaning that if insemination takes place too early, the sperm is aged and by the time ovulation occurs it cannot fertilize the ovum and if insemination takes place too late, the egg is aged and fertilization and formation of a viable embryo is not likely. Indications exist that, in practice, an enormous variability exists in the timing of insemination relative to ovulation (Roelofs *et al.*, 2005).

There are various reports that indicate low rate of AI service in cattle is mainly due to problems of estrus detection. When natural service is used there are no problems, since a cow will only stand for the bull when she is in estrus, and under free-range conditions a cow may be served several times at each estrus. Several studies (Arthur, 2001; Jane *et al.,*2009; Morrell, 2011) report on the correct timing of AI which is a dependent upon true, accurate and early identification of estrus, the accurate identification of the individual animal and informing the inseminator at the correct time. A cow that is first seen in estrus in the morning is usually inseminated in the afternoon of the same day, whilst a cow that is first seen in estrus in the afternoon is inseminated early the next day (Arthur, 2001). A protocol has been developed using GnRH and PGF2α that synchronizes the time of ovulation within an 8-h period (24 to 32 h after the second injection of GnRH) with PR/AI similar to am/pm breeding. This precise synchrony of ovulation allows for an effective test to determine the optimal time of AI in relation to ovulation (Roelofs *et al.*, 2006).

2.2.3. Intrinsic factors related to the cow

Postpartum problems including endometritis have been reported to have a negative effect on fertility i.e. first service conception rate is poor and primiparous cows have higher conception rates than older cows under use of two timed breeding protocol (Tenhagena *et al.*, 2001; Rahim and Asghar, 2007). Reproductive health‘s of the cow particularly uterine environment is of paramount importance in the success of AI. High reproductive efficiency is dependent on obtaining normal uterine involution, early resumption of ovulation, high efficiency of estrous detection and high conception rates per service (Tenhagena *et al.*, 2001; James, 2006). The presence of uterine infection prolongs uterine involution and resumption of ovulation. Cows with uterine infection in the early postpartum period generally have lower conception rates at subsequent breeding. Studies confirm that even mild uterine infections adversely affect conception rates (O'Connor, 1993). Rahim and Asghar (2007) described on his finding that health and reproductive disorders to post-calving seriously affect conception rate. Calving and post-calving reproductive disorders seriously affect conception rates. Thus, the key to maximizing conception rates must lie in the prevention of disorders, not treatment after they have occurred. Some evidences indicate cows suffering from metabolic disorders, like milk fever, may have a higher incidence of reproductive disorders and lower conception rates (Arthur, 2001; Rahim and Asghar, 2007).

2.2.4. Early embryonic loses

Reproductive failure in inseminated cattle results from poor fertilization and embryo survival (Santos *et al*., 2004). Reduced conception rates could be due to early embryonic mortality which contributes to reproductive inefficiency in lactating dairy cows. Fertility assessed at any point during pregnancy is known to be a function of both conception rate and pregnancy loss (Arthur, 2001; Chebel *et al*., 2004). Pregnancy loss in lactating dairy cows is greater than that in dairy heifers, though the specific factors responsible for early embryonic loss in dairy cows are not known. Different studies reviewed that early embryonic loss in cattle is difficult to study because no sensitive test similar to that used for women and mares exists (Romano *et al.,* 2007). According to Romano *et al*. (2007) rate of embryo/fetal death between days 30 and 60 was 14.0% which may be potential reason for repeat breeding (Singh *et al*., 2005), and on the other hand the fertilization rate after AI in beef cows is 90%, whereas embryonic survival rate is 93% by Day 8 and only 56% by Day 12 post AI. In dairy cattle, only 48% of embryos were classified as normal on Day 7 after AI. Thus, substantial pregnancy loss probably occurs within two weeks post AI (Arthur, 2001; Santos *et al*., 2004). Rectal palpation at early stage of conception by non practitioner could be also for loss of early pregnancy (Romano *et al*., 2007). So that the use of transrectal ultrasonography permits an earlier pregnancy diagnosis than palpation per rectum, gives immediate information to confirm pregnancy as well as assess embryo/fetal viability, and reduces the number of false positive diagnoses and false negatives when palpation per rectum is used (Romano *et al*., 2007). On the other hand conception rate to first service is the combined consequence of fertilization, early embryonic, late embryonic and fetal development, and each of these steps in establishing pregnancy may be affected. Grimard *et al.* (2006) cited that fertilization failure and early embryonic loss, late embryonic/fetal loss, and late abortion represent 20 - 45%, 8 - 17.5% and 1 - 4% of pregnancy failure, respectively.

2.2.5. Factors related to management

Several factors related to management play roles in successful pregnancy among which nutritional management contribute the largest proportion. Fluctuation in season which have effect on availability of feed, high environmental temperature and other environmental factors (Haugan *et al*., 2005) cause stress and the challenge of high disease risk in cross breed cows that contribute to high number of services per conception, late age at first calving and first service, and longer calving interval (Mukasa-Mugerewa, 1989).

According to Smith (1982) and Anzar *et al*. (2003) energy status is generally considered to be major nutritional factor that influence reproductive performance. Also total dietary intake can affect fertility, both at the level of the oocyte and embryo, which means it is important to differentiate between optimum conditions for follicle growth and embryo survival. Nutrition has effect on quality of follicle, oocyte and embryo (Dhaliwal *et al.*, 1996; Maurice *et al*., 2003; Funstone *et al*., 2009). Maurice (2003) briefly described the blastocyst formation as a key developmental process in the growth of an embryo. Dietary intake and diet type can alter the expression of transcripts of genes involved in early embryo development. Nutrient requirements for optimum follicle growth and embryo development may be quite different. Hence, the importance of diet around the time of mating and in particular the significance of extreme underfeeds post mating in regulating pregnancy rate becomes evident.

Xu *et al*. (2010) reported that the peak of embryo death occurs during the first month of pregnancy and controlled feed intake is important to reduce mortality of embryos. He has demonstrated that a diet consumed before mating has a major impact on embryo survival and litter uniformity in blastocyst size in pigs. As mentioned by Robinson *et al.* (2006) the impact of nutrition on embryo survival in ruminants extends beyond the supply of essential nutrients and the modification of the hormones and growth factors that influence embryo development.

Housing conditions can also have an effect on the distribution of heat during a 24-hour period. Estrous behavior expression at any housing arrangement that allows cattle to interact throughout the day provides more opportunity for mounting and standing behavior to be expressed which enable to identify estrous cow easily. Especially on cattle housed in tie-stall or stanchion barns must be turned out in order for this behavior to be expressed. O̒connor (1993) reported under research conducted on high-producing Holstein cows to what extent slippery footing surface could have affected expression of estrus. The result showed that mounting activity occurs more frequently when cows are on soil rather than concrete. When five estrous cows were individually presented with an opportunity to spend time on soil or on concrete in the presence of a tied cow which was either anestrous cow or a cow not in heat, the test cow spent an average 70% of the time on ground (O̒connor, 1993) and 73% (Morrell, 2011). The test estrous cow mounted more frequently when a tied estrous cow was on soil rather than on concrete (O̒connor, 1993).

2.2.6 Factors related to semen quality

Considering the economic investment in semen and other inputs, success must be judged on the basis of pregnancy rate to the first AI. Also, a good first service pregnancy rate response usually signifies good conditions for second service (Saacke, 2008). Additional key factors to be considered as impacting pregnancy rate to first service are semen quality (primarily dependent on choice of bulls). In most breeding strategies, whether estrous synchronization is employed or not, semen quality is one of a critical point to a successful pregnancy. The nature of sub fertility due to the male is proving as complex as that due to the female.

Saacke (2008) emphasized that the importance of semen handling and placement to achieve threshold or above threshold number of sperms to the ovum necessary to maximize both fertilization rate and embryo quality. Sprmatozoa become very sensitive to any form of stress in their environment in vivo as well as in vitro. As a result, fertility from frozen thawed semen is poorer than that obtained from fresh semen (Lemma, 2011; Arthur, 2001). For this reason, proper evaluation of the post thaw quality of spermatozoa is of utmost interest for AI industry to obtain information on the fertilizing capacity of the cryopreserved semen. Many tests of sperm motility, morphology, acrosomal status, defective sperm organelles and DNA, and metabolism have been correlated with fertility (Lemma, 2011).

2.2.7 Factors related to insemination techniques

One of the most significant contributions to the successful application of AI in cattle breeding has been made by the highly trained inseminator (Arthur, 2001). The efficiency of cow insemination depends, among other factors, on the ability of the inseminator to deliver the semen to the appropriate site in the reproductive tract at the appropriate stage of estrus. However, there has been a tendency to adopt routine insemination techniques and to ignore inseminator-related factors that can dramatically affect fertility. Although professional inseminators palpate the reproductive tract of numerous cows every day, most are not trained to examine the uterus and ovaries. This poses a serious practical limitation to the success of AI (López-Gatius, 2011). Animals showing signs of true heat should inseminate using frozen semen thawed at 37 ^{0}C for 30 seconds (Iftikhar *et al.*, 2009).

Professional technicians are more successful at insemination than inexperienced ones, indicating that selection of a qualified inseminator to be an important element in the success of AI program and regular practice at inseminating is required to maintain high conception rates. Citation evidenced in Gebremedhin (2008) shows that the site of semen deposition has been an important factor in the success of AI in cattle. The deposition of semen in the uterine body resulted in a 10% higher non-return rate than did cervical deposition and an increase in the conception rate has been reported when semen was deposited in the uterine horns rather than the uterine body. Cows are inseminated just into the short uterine body (O`connor, 2003). Insemination into the cervix produces a lower fertilization rate, while insemination deeper into the uterus runs the risks of either inseminating into the uterine horn contra lateral to the ovulation site, or scaring the endometrium with the tip of the insemination catheter. Reduced fertility is the consequence of both of the later two errors (Arthur, 2001; O̒connor, 2003). In the early days of AI there was controversy among researchers about the proper site for semen deposition. A study conducted in Canada cited by O̒connor (2003), provided evidence that fertility was highest when semen was deposited in the uterine body. Researchers currently are re-examining insemination technique to determine the proper site of semen deposition (O̒connor, 2003). Failure to understand the anatomical and functional relationships among the various tissues and organs of the reproductive system may lead to consistent insemination errors (Arthur, 2001; O̒connor, 2003).

The ability to perform an intrauterine insemination in cattle means that a relatively low dose of sperm is required to achieve acceptable pregnancy rates. Typically, of the 20–30 million sperm that are required in each insemination dose, 6-7 million survive freezing, that is generally regarded as the minimum dose compatible with acceptable fertility (Arthur, 2001). Regarding to depth and time of insemination (Arthur, 2001; Morell, 2011) recommends that very deep insemination can enhance sperm delivery. However, site of insemination was found to make only small increases in sperm per egg. Deep insemination should be used only when the sperm dose is below threshold, or if sexed semen is being used. Also hygiene, thawing methods, temperature maintenance between thawing to insemination do play a factor in achieving pregnancy (Morrell, 2011). Rectal palpation and ultrasound examinations should be considered safe procedures when performed correctly, and recent evidence gives no indication that ultrasound examination is detrimental to the embryos.

2.3. Estrus Induction/Synchronization in Improving Success of AI

Estrus induction is the process of bringing female animals to heat state of those having preovulatory follicular activity (Jane *et al.*, 2011). Hormonal interventions have been used to increase the probability of estrous detection and leads to timely insemination to increase pregnancy rates of dairy cattle (Lucy *et al.*, 2004). The hormones used to control the estrous cycle mimic the reproductive hormones found within the normal cow. Most estrous induction/synchronization systems employ a method for controlling follicular wave development, promoting ovulation in anestrous cows, regressing the corpus luteum in cyclic cows, and synchronizing estrus and (or) ovulation at the end of treatment. In most herds, a non-intervention period is practiced where postpartum cows are observed estrus (Day, 2004; Lucy *et al*., 2004; Holm *et al*., 2008). Cows not observed in estrus are then treated (Lucy *et al*., 2004; Jane *et al*., 2011). Detection of estrus requires the presence of infertile (e.g. vasectomised) males, or the timing of estrus must be controlled by pharmacological means such as estrus synchronisation/induction (Arthur, 2001). Estrus induction/synchronization has been primarily carried out by the use of commercially available prostaglandins (PG) such as dinoprost (lutalyse) or cloprostenol (estrumate). These products are from a class of hormones which generally cause regression of the corpus luteum, subsequent manifestation of estrus and ovulation within 2 to 5 days (Holm *et al*., 2008). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) causes lysis of the corpus luteum (CL) in bovines from day 5 until day 17 of the estrus cycle, and can be used to synchronize any female‘s estrus cycle from day 7 onwards. The time from treatment to induced estrus is inconsistent, but generally varies from 2 to 5 days in heifers, depending on the stage of the follicular wave at the time of treatment (Holm *et al*., 2008).

3. MATERIAL AND METHODS

3.1 Study Area

The study was conducted in Siltie zone of Southern Nation Nationality and People Regional State (SNNPRS) comprising 8 districts. The zone‘s main town (Worabe) is located on the road to Hossana just 172 km from Addis Ababa. According to SZFED (2011) censuses the human population of the zone is 831,573 of which 51% are male and 49% female. The settlement is 95.5% and 4.5% living in rural and urban areas, respectively. Agriculture is the predominant economic activity there by the largest contribution of the zone‘s GDP representing around 90% of people‘s main livelihood in form of mixed farming.

Siltie zone has different agro-ecological zones ranging in altitude between 1500 and 3700 masl; namely highland (Dega) representing about 20.5% and midland (Woynadega) comprising 79.5% of the zone. The average temperature range from 12 - 26 ^{0}C and the average annual rainfall ranges from 780 - 1818mm. Wheat, Maize, Sorghum; Enset and Vegetables are the major crops. Different types of livestock populations found in the area. From the total of 10,543,129 livestock population found in the region, 17% (1,793,033) are found in Siltie zone. Out of this 29.3% (525,178) are cattle of which 99.5% are local breeds with the remaining 0.5% (2,556) representing cross breeds (SZFED, 2011). Cattle are used as a source of draft power, manure, milk, meat and source of income from sell of animals. The main livestock feed sources in the area are natural pasture and crop byproducts including hay and straw (barley, teff, and wheat).

3.2 Study Animals

All animals in the district were grouped in to two: the first group was included in the survey study and the second group included in the experimental study. All 17 PAs in the district were included in the study for sampling. The entire study population in these PAs was divided in to two strata. The first stratum contained owner‘s in 15 PAs that were purposively sampled based on their free access to government delivered AI service. The second stratum included the remaining two PAs to avoid overlapping and was destined for the experimental study. All the study animals were vaccinated against black leg, anthrax and pasteurollosis. In the first group a total of 275 cows belonging to 247 dairy owners were selected and the second group contained sample size of 94 cows. Body condition of experimental animals was determined on 1- 9 scale using method described in Nicholson and Butherworth (1986) and animals were divided in to three groups (1-3 = poor, 4-6 = moderate and 7-9 = fat body condition). Animals with very poor BCS (< 3) were not selected for insemination during sampling since estrous expression is highly influenced by body condition (Lucy *et al*., 2004). History of each cow‘s body condition, health status, feeding status and other related information have been taken during questioner survey (Annex I and II).

3.3 Study Design

3.3.1. Study on factors associated with AI delivery system

Study on the factors affecting AI delivery were conducted using questionnaire survey, personal interview of dairy owners and professionals, direct observation and record analysis. Data were collected from dairy owners, AI technician, veterinarians and record of MoA. Information regarding the farming system, data on the animals such as presence of previous calving problems and related factors were included in the survey. Awareness of owners was further subgrouped in to Good awareness (owners with good knowledge of estrus signs, make decision for AI and bring their animals for AI at the appropriate time based on the am-pm rule) or Poor awareness (owners having little or no knowledge of estrus, either do not use AI or, if so, bring their animals outside the am-pm rule). Moreover, most common sign recorded/observed, duration of estrus to AI, number of previous AI, distance of service center at the time of AI, service per conception, skill of the AI technicians (level of training, on-job training, year of service, condition of the service delivery (mobile or stationed) and condition of pregnancy diagnosis have also been included in the survey. Duration to AI was further sub-grouped as Before time (0-7 hrs), On time (7-12 hrs) and After time (>12hrs). Further, parity, BCS, and major estrus signs exhibited were also recorded.

Information regarding the source of semen including storage and semen handling facilities, availability of transport system, availability of finance and logistics for procurement of materials for AI (liquid nitrogen, fuel, spare parts etc) and cost were recorded. Further, time and method of pregnancy diagnosis, decision in unsuccessful AI, record taken and feedback to AI centers have been collected. All animals having parity numbers from 1 to 4 and inseminated at the AI station during study period were considered and pregnancy diagnosis was carried out after 60 days post insemination.

3.3.2. Application of estrus induction and insemination

A total of 94 local zebu and cross breed Holstein Frisian cows were selected. Animals were selected based on reproductive performance (proper cycling, parity=1-4, Calving to first service= 3-24 months, and NSPC=1-3) and absence of history of both general and reproductive health problems. The reproductive status of the animals was evaluated and recorded from history, clinical examination and rectal palpation before hormone treatment. Only cycling, non-pregnant cows carrying a corpus luteum have been chosen for the study. Each cycling cow was given a single intramuscular injection of 2 ml of synthetic prostaglandin (cloprostenol sodium or estrumate; manufactured by Germany, Schering-tlough ltd company) according to a previously described method (Rasby and Deutcher, 2010; Figure 1). Cows were meticulously monitored for manifestation of estrus signs (mounting, bellowing activities and mucus discharge) for about 5 days. All cows manifesting two or more signs of estrus were then inseminated based on the am/pm guideline using semen procured from NAIC. The inseminated cows were further monitored for evidence of estrus, morning and evening until the beginning of presumed next cycle. A single inseminator was assigned to carry out all the inseminations using the recto vaginal method.

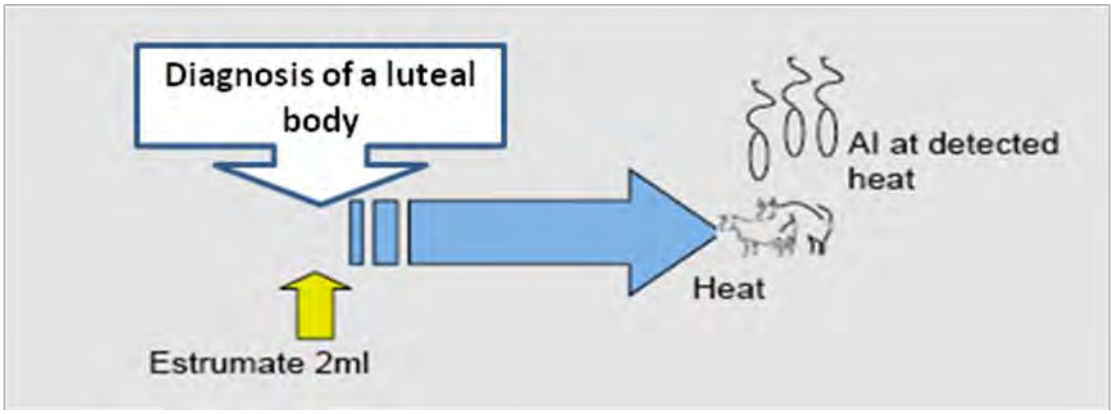

Figure 1: Single injection of prostaglandin analogue (estrumate). Source: Rasby and Deutcher (2010).

3.3.3. Data analysis

All the data was entered in to Microsoft excel sheet. Analysis of the data were performed by using a computer software SPSS for windows (version 16.0, USA). Data was summarized using descriptive statistics (means, standard errors, proportions, minimum and maximum values). The fixed effects of BCS, breed, estrus induction, and duration to AI on pregnancy rate were computed using student t-test and ANOVA. Proportions were compared using Chi-square test. The relationships between variables were computed using Pearson correlation. The level of significance was held at $p<0.05$ to show statistically significant differences among variables.

4. RESULT

4.1. Status of the conventional AI delivery system

In study site there were eight AI stations for the whole of Siltie zone consisting more than 525,178 cattle. The AI program is a government run activity under the ministry of Agriculture established with nine (9) mid-level professionals (inseminators) delivering the service for eight districts within the zone. Semen and liquid nitrogen were known to be procured from the Wolkite (140km) and Wollaita Sodo (150km) Liquid Nitrogen and Semen Dispatch Centers on demand basis. AI is delivered at a fixed place within each district's veterinary clinic and no transport facility is available to deliver AI on site/farm. Animals showing estrus travel between 0.5km within the respective towns and up to 10 km from around the periphery PAs. AI service cost about 2 birr/cow, whereas the decisions regarding the type of bull/semen is made by the inseminators. Observation of the estrus signs and bringing the animals for AI solely rests on the dairy owners however; inseminators do not countercheck signs of estrus before insemination. The total numbers of cows inseminated using the conventional method were 275 during the study period from October 2011 to February 2012. Pregnancy was confirmed rectally after two months. Summary of information regarding the management and AI related parameters collected during the questionnaire survey is presented in table (4).

Table 4: Summary of AI delivery-system-parameters during the questionnaire survey (n=275)

Parameters	Animal Category	Value	Proportion
Owner experience	Good experience	165	60%
	Poor experience	110	40%
Breed	Zebu	203	74%
	Cross	72	26%
BCS	Poor	49	17.8%
	Moderate	226	82.2%
NSPC (for pregnant)		2.34±0.05	
Average distance travelled for AI	Within the town	1.5km	-
	From surrounding PA	4.5km	-

NSPC= Number of service per conception

Awareness of owners on estrus manifestation, decision made for AI and time at which animals were brought for insemination had a direct impact on success of the AI. Comparison of pregnancy rate in cows population belonging to owners with good and poor awareness showed a significant difference ($p<0.05$; Table 5).

Table 5: Analysis of the role of awareness of estrus signs and time of AI by owners on pregnancy success

Awareness of owners	Number of cows	Pregnancy result[%]		χ^2	p-value
		Non pregnant	Pregnant		
Poor awareness	110	79(71.8)	31(28.2)		
Good awareness	165	78(47.3)	87(52.7)	16.232	0.00
Overall	275	157(57.1)	118(42.9)		

The most common estrus signs observed by the owners are presented in table (6). There was a strong association between manifestation of vaginal mucus discharge and higher success of pregnancy.

Table 6: Most common estrus signs detected by owners and associated pregnancy rates in animals using the conventional AI service (n=275)

			Pregnancy result		
Estrus signs	**Status**	**N**	Non pregnant	Pregnant	**P-value**
Bellowing	Did not bellow	41	25	16	0.586
	Bellowed	234	132	102	
Mounting	Not mounted	39	28	11	0.045
	Mounted	236	129	107	
Mucus discharge	No mucus discharge	101	97	4	0.00
	Mucus discharge	174	60	114	

Pregnancy success was also influenced by the breed of the cow and frequency of presentation for AI. A higher success of pregnancy was observed among the cross breed animals compared to the local zebu cows (Table 7).

Table 7: Cows that received AI through the conventional method at the time of questionnaire survey

Breed	No. cows	AI after last calving				Pregnancy rate
		First time Service	Second time Service	Third time service	≥4 time service	
Local	203	75	61	64	3	70 (34.4%)
Cross	72	10	21	39	2	48 (66.7%)
Total	275	85	82	103	5	118 (42.9%)

Clinical examination of animals before insemination did encounter different kinds of reproductive health problems such as dystocia (n=6/275), prolapse (n=7/275), RFM (32/275), abortion (n=3/275) and vaginal discharge (n=1/275). Pregnancy rate in cows without disorder were higher than that of cows with reproductive disorder (Table 8).

Table 8: Reproductive disorders encountered during the previous pregnancy and pregnancy rate

Pregnancy diagnosis result	Status of Disorders	
	Without disorder (n=226)	With disorders (n=49)
Non pregnant	50.4%	87.7%
Pregnant	49.6%	12.4%

There was a substantial association between the duration to AI and pregnancy rate (Table 9). Animals that were brought for AI on time had the highest success of pregnancy.

Table 9: Descriptive statistics of conventional service and its pregnancy result

Category of AI time [hrs]	N	Mean (±SE) duration to AI [hrs]	Range [hrs]	Pregnancy rate [%]
Before time (0-6hrs)	116	3.74±0.15	1-6	24.1 (28/116)
On time (7-12hrs)	104	9.03±0.14	7-12	83.6 (87/104)
After time (>12hrs)	55	15.80±0.32	13-26	5.3 (3/55)
Overall	275	8.15±0.29	1-26	42.9 (118/275)

The mean (±SE) BCS for the cows under the conventional AI system was 3.4. Cows were further sub grouped in to moderate (4-5) and poor body condition (score 3) to assess the effect of BCS on pregnancy rate. Animals with moderate body condition had a significantly higher pregnancy rate (49.5%) than those with poor body condition (12.2%) (Table 10).

Table 10: Effect of body condition score on pregnancy rate

BCS	N	Pregnancy result: Non pregnant	Pregnancy result: pregnant	χ^2	P-value
Moderate BCS	226	114	112		
Poor BCS	49	43	6	22.89	0.00
Total	275	157	118		

4.2 Artificial estrus induction and AI

Estrus induction was carried on both zebu and cross breed animals that were also under similar management system with the cows considered for the conventional AI. Average time of induction to first estrus and standing heat to AI were 57.49±1.9hrs and 8.62±0.15hrs respectively. Estrus response rate was 93.6% (n=94) and summary of estrus signs, BCS and the respective pregnancy rate recorded are summarized in table (11). Similar estrus signs were overtly manifested in induced animals.

Table 11: Description of estrus signs, BCS and respective pregnancy rates for induced and non induced cows that received AI for the first time (First service)

Parameters	Induced cows (**n**=77)	Pregnancy rate [%]	Non Induced cows (**n**=85)	Pregnancy rate [%]	Total (**n**=162)
Estrus signs					
Bellowing	51	49	71	28.1	122
Mount	45	44.4	72	25	117
Mucus	39	64.1	42	54.7	81
Body condition					
Poor	17	0	20	15	37
Moderate	60	61.7	65	32.3	125

As shown in the figure (2) dairy herd owners mostly bring their cows Before time compared to those under the estrus induction regime. Cows were brought for AI immediately or within few hours of estrus or very late after estrus signs subside. Owners were known to be reluctant to stay until the cows show standing estrus.

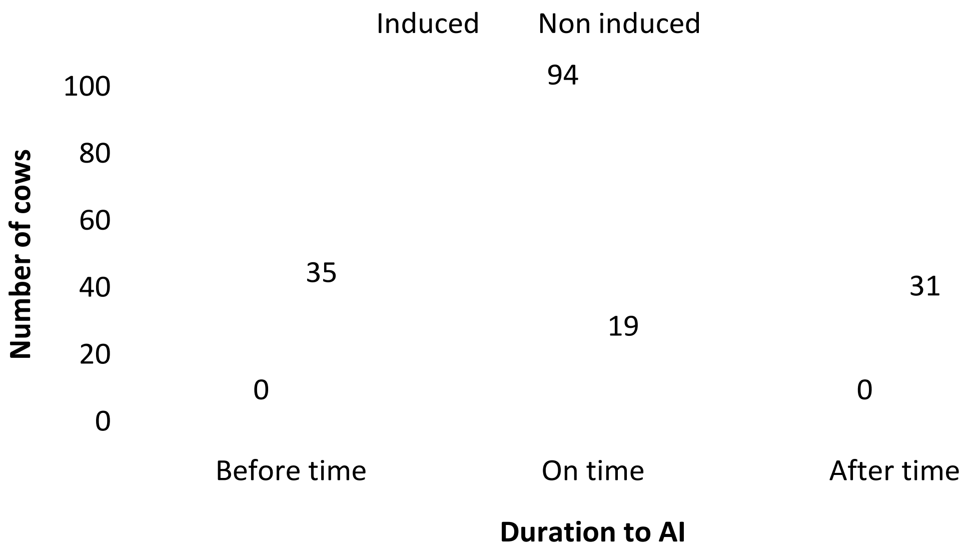

Figure 2. Description of duration to AI after estrus detection for induced cows under the experiment and non induced cows under the conventional AI

The role of each estrus sign manifested in the success of pregnancy regardless of estrus induction procedure was computed. There is a significant difference ($p<0.05$) in pregnancy rate among cows manifesting different estrus signs with the highest success being recorded in cows that showed vaginal mucus discharge (Table 12).

Table 12: Effect of estrus signs on first service pregnancy rate regardless of the estrus induction procedure (n=162)

Estrus signs	Status	Result Pregnancy rate [%]	χ^2	p-value
Bellowing	No bellowing	40	0.124	0.724
	Bellowed	36.9		
Mounting	No mounting	51.1	4.806	0.028
	Mounted	32.5		
Mucus discharge	No mucus	16.0	32.211	0.000
	Mucus discharge	59.3		

4.2.1 Timing of insemination

The duration to AI was similarly categorized as Before-time, On time and After time, and pregnancy rate was compared among different duration to insemination (Figure 2). Basically, zebu breeds were known to have short period estrus sign and a weak estrus manifestation. First service pregnancy rate was 28.2% (24/85 rectally diagnosed) for non induced cows while it was 48.1% (37/77 ultrasonically diagnosed) for induced cows. There is a significant difference ($p<0.05$) in pregnancy rate between induced and non induced cows with pregnancy being 1.70 times higher for induced cows. Pregnancy rate for cows brought on time was also known to be significantly higher ($p<0.05$) compared with those inseminated Before-time or after time (Figure 3).

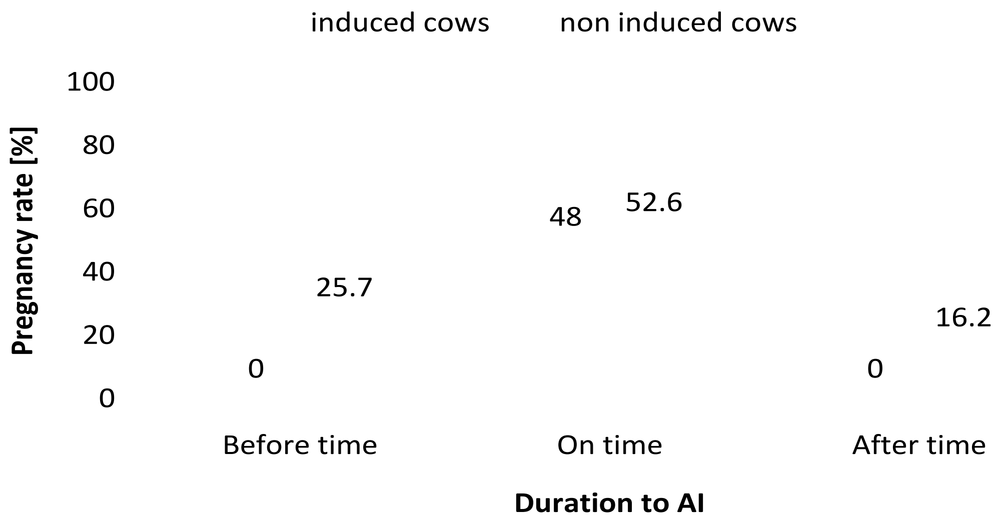

Figure 3. Relationship between duration to AI after estrus detection and pregnancy rate in induced and non induced cows

Owners experience did have effect on pregnancy rate of induced animals. Owners living in and around the towns had a relatively better concept of estrus signs, use of AI and used improved breeds compared to owners living in remote PAs. Animals owned by owners with good awareness had a significantly higher pregnancy rate ($p<0.05$; 55.9%) compared to those owned by owners with poor estrus awareness (27.1%). The overall mean (±SE) number of service per conception was 2.34±0.05 for animals considered under the conventional AI delivery. Overall higher pregnancy rate was found in cross than local breed induced cows 50% and 35.5%, respectively which was also statistically significant ($p<0.05$) though parity had no significant effect on success of pregnancy. First service pregnancy rate was also significantly different ($p<0.05$) between induced and non induced cows, however, there was no breed difference in pregnancy rate for induced cows (Table 13).

Table 13: Comparison of first service pregnancy rate between induced and non induced cows (effect of estrus induction on pregnancy rate) and between zebu and cross breed animals.

Breed	Non induced cows			Induced cows		
	Nº of cows at first time AI	Pregnancy rate [%]	p-value	Nº of cows at first time AI	Pregnancy rate [%]	p-value
Zebu	75	18 (24%)	0.018	62	30 (48.4%)	0.905
Cross	10	6 (60%)		15	7 (46.7%)	
Total	85	24 (28.2%)		77	37 (48.1%)	0.009

Number of services per conception is the number of services required for a successful conception and in the current study mean of services per conception was found to be 2.34±0.05.

5. DISCUSSION

The overall pregnancy rates obtained in non induced dairy cows 42.9% (n=275) is far lower than different reports (Mukasa-Mugerwa 1989; Shiferaw *et al.*, 2003; Mureda and Mekuriaw, 2007) and what is acceptable standard (Falvey and Chantalakhana, 1999) for an economically viable production indicating the presence of a severe performance problem (Rogers, 2001). The present finding is also by far lower than the previous 84.7% reported by Shiferaw (2002) and 63-71% reported by Arthur (2001). Further, the first service conception rate which is 28.2% is much lower than previous reports in Ethiopia and elsewhere (Mukasa-Mugerwa *et al*. 1991a; Shiferaw, 2002; Chaundhari and Sabo, 2006). In general, low fertility rates of cattle in the tropics compared to temperate regions are known to be related to environmental differences including inadequate nutrition, prevalence of diseases as well as the interaction between genotype and environment (Mukasa-Mugerwa 1989). The relatively higher service per conception and lower pregnancy rate in the present study could also be related to nutritional status (as manifested by the poor BCS) that is directly related to estrus manifestation (Dhaliwal *et al*., 1996; Anzar *et al*., 2003; Maurice *et al*., 2003; Haugan *et al*., 2005). Though cows with BCS 3 or greater were selected for this study, the comparison of pregnancy rates between cows with poor and moderate BCS clearly revealed the influence of nutritional status. Good BCS during the mating period has been confirmed to have a positive impact on conception rate (Mukasa-Mugerwa, 1989: Kaziboni *et al*., 2004). The current result also closely agreed and strongly associates with this finding. Moreover, Shiferaw *et al*. (2003) indicated an increase in pregnancy rate with increasing BCS.

The mean number of services per conception obtained in the study was 2.34± 0.05. The result is higher than Falvey and Chantalakhana (1999) <1.8, 1.74-1.8 by Tegene *et al*. (1981), 1.75 by Shiferaw (2002) and 2.0 ±0.1 by Nigussie *et al*.(1998). Services per conception greater than 2.22 indicated that presence of severe herd fertility problem (Rogers, 2001). Previously no research has been conducted on efficiency of AI in and around the study area, though the report released by NAIC (1995) indicated that the average services per conception in SNNPRG zones such as Sidama 1.9, North Omo 1.7 and Hadiya 2.22 and the present study. The value in the current study is much closer to the cases of Hadiya. Also the service per conception obtained (2.34) was by far higher than the result 1.4±0.6 found by Sovani and France Chini (1998) in cross breeds. This could be due to the estrus detection problem. Also the variation might be due to the difference of breeding system used (Mukasa-Mugerwa, 1989) and other related factors as mentioned above.

The fact that only few AI technicians are serving large population of cattle, semen is procured from Wolaita and Wolkite; and cows are travelling up to 10km to get the service further constrained by poor awareness of owners about AI are contributors of the poor efficiency of AI in the zone. Such logistic and system related constraints have been previously discussed by different reports (Tegegne *et al.*, 1989; Belihu, 2002, Gebremedhin, 2008). An important finding to note was the difference in pregnancy rate achieved in cows whose owner had better awareness (55.9%) compared to those having poor awareness (27.2%). Shiferaw *et al.* (2003) report supported this idea as rearing improved breeds and maintaining optimum body condition to be consistent with awareness and experience of the dairy owners. Such significant difference in pregnancy rate between the two kinds of owners in the current study could be attributed to the requirements of meticulous observation of estrus for best success of AI as well as related reproductive management of breeding animals (Arthur, 2001; Bekana *et al.*, 2005; Iftikhar *et al.*, 2009). A good knowledge of the cows estrus behavior and the estrus to ovulation interval is known to be essential for estimating the best time for AI (Pinheiro *et al.*, 1998; Barros *et al.*, 2006; Reolofs *et al.*, 2010). The higher rate of pregnancy emanating from comparison of pregnancy rate base on the time of presentation for AI (Before-time, On-time and After-time) in the current study, clearly shows the influence of timely insemination as also confirmed by several authors (Pinheiro *et al.,* 1998; Arthur, 2001; Ball and Petters, 2004; Reolofs *et al.*, 2010).

A realtively higher pregnancy rate that was significantly different from the Zebu breeds(35.5%) was obtained in cross bred animals (50%) this study. This is inline with the report of Mukasa-Mugerwa (1989) and Reckwot *et al.* (1999) in zebu breeds. Breed differences in pregnancy rate have been established by different authors primarily attributed to shorter and less intense estrus in Zebus which might lead to incorrect timing of insemination (Tegene *et al.*, 1989; Mukasa-

Mugerwa *et al*., 1991; Pinheiro *et al.,* 1998; Arthur, 2001; Ball and Petters, 2004; Reolofs *et al*., 2010). As accurate timing of estrus is profoundly important for successful pregnancy, some studies indicated that up to 15% of the cows presented for AI might not in estrus (Chaundhari and Sabo, 2006). Good estrus detection (>90%) is essential to high cow fertility to AI. Faulty heat detection, resulting in failure to present cows for service at the correct time, is the biggest single cause of "low fertility" in herds inseminated by AI. Great vigilance is needed to detect 90% of cows in heat. Many farmers miss 20-40% of their cows in estrus whereas some miss >50%, especially if labor is scarce (Rogers, 2001). For conception to occur, AI must be served at the right stage of the estrus cycle. Failing to do this either due to poor knowledge of estrus sign, or poor estrus manifestation would result in failure of pregnancy (Rodriguez-Martinez, 2000; Cartmill *et al*., 2001). However, estrus signs observed in the current study were similar to other studies (Arthur, 2001). In reality, it is confirmed that there is a great variability in the timing of insemination relative to the time of ovulation (Roelofs *et al*., 2005).

Apart from estrus and management related factors, different kinds of reproductive disorders found in the current study have also been reproted elsewhere. When pregnancy rate was compared between cows with previous history of reproductive disorder and those without, a significant difference was found. The negative effect of different kinds of reproductive disorders on both conception rate and pregnancy rate have been established in dairy animals (Robert, 1986; Bekana *et al*., 1987; Arthur, 2001; Tenhagena *et al*., 2001; Rahim and Asghar, 2007). This again proofs the significance of performing regular gynecological examination as part of the reproductive herd health management before animals are allowed to be breed. Conception rates are known to be adversely affected even by the mildest uterine infections (O'Connor, 1993; Tenhagena *et al*., 2001).

In the current study, estrus induction is considered to elude the need for estrus detection and thereby positively impact the development of AI through successful pregnancy. A study by Bekana *et al*. (2005) 80% and Reckwot *et al*. (1999) using PGF2α single injection with three different trials was known to give estrus response rates of 90.5% which is closely similar with current study (93.6%). The uses of hormones have been indicated to increase the probability of estrous detection, and to increase pregnancy rates of dairy cattle under a variety of management systems (Lucy *et al*., 2004; Jane *et al*., 2011). Prostaglandins used for this purpose are generally known to cause regression of the corpus luteum and subsequently lead to the expression of estrus and ovulation within 2 to 5 days after their administration (Roelofs *et al*., 2005; Holm *et al*., 2008). Pregnancy rate from 61.4% to 65% in cycling and up to 47% in anestrous cows have been reported after a single injection of PGF2α (Reckwot *et al*., 1999; Perry, 2005) The pregnancy rate for induced cows in the current study (48.1%) though highly improved compared to the non-induced ones (28.2%) was still lower than the optimum or those given in other reports (Falvey and Chantalakhana, 1999; Arthur, 2001; Shiferaw, 2002; Mureda and Mekuriaw, 2007). Woldu (2011) reported an overall conception rate of 48.3% in crossbred and indigenous Zebu cattle types in Arsi area. Sovani and Franceschini (1998) from Debre Zeit ILCA station reported a closely similar conception rate of 59% for zebu breeds. On the other hand, the present finding is within the range of 40-60% previously reported studies in Ethiopia (Gaines, 1989, Shiferaw, 2002) and elsewhere (Arthur *et al*., 1989; Mukasa-Mugerwa, 1989). The difference among the different reports could be attributed to a multitude of factors such as estrus detection, body condition, skill of the inseminator, service time after onset of estrus, semen quality and breed of animals.

Particularly the lower result in the current study might be associated with poor heat detection and poor body condition of animals in the study area at the time of AI. Moreover, AI conducted at approximately 12 hrs after onset of standing heat potentially has lower fertilization rate with increasing embryo degeneration (Roelofs *et al*., 2010; Dalton, 2011). The estimated time required for sustained transport of viable sperm to the oviducts is between 6 to 12 hours. The functional viable lifespan of sperm in the female reproductive tract has been estimated at 24 h (Nebel *et al*., 2000; Woldu *et al*., 2011), while the optimal window for fertilization of the ovum is estimated to be less than 10 h indicating the need for optimal time of AI. Most animals under the experimental induction were also poor in their body condition. Seasonally fluctuating forage availability coupled with high ambient temperature have been known to affect success of pregnancy (Smith 1982; Anzar *et al*., 2003; Haugan *et al*., 2005). Particularly, estrus manifestations have been known to be short, erratic and mostly less evident or silent further requiring a meticulous observation and timely insemination to result in successful pregnancy.

A follow-up study of non induced cows that failed to be pregnant revealed that 16.3% of them received ≥3 services. Higher number of service per conception often leads to repeat breeding which has been reported to range between 10.2 and 13.7% (Robert, 1986). As indicated by Hafez (1993) the rate of repeat breeders is higher in dairy herds using AI. Arthur (2001) associated this case with early embryonic mortality, poor semen quality, poor insemination techniques, uterine infection and related factors. Also Shiferaw (2002) detected high proportion of reproductive abnormalities as having important contributions to reproductive inefficiency.

6. CONCLUSION AND RECOMMENDATIONS

The overall pregnancy rate found in the current study is by far lower than the optimal rate expected in a profitable farm. Though, poor pregnancy rate are multifactorial the finding of poor body condition resulting from seasonal availability of pasturage on which most of the cows depended have contributed much. Even with the best feeding management, estrus detection remains to be the biggest single cause of lower fertility of a herd. In the conventional AI system, most dairy owners did not have a good knowledge of estrus signs, even when they do, did not bring their animals at the right time for insemination. The fact that insemination centers are mostly at highest distance from farmers of the surrounding PAs has also contributed to delay in the proper timing of insemination. Hence, in the conventionally used AI delivery system of the study area, it can be concluded that the key problems to the success of AI program are attributed to poor nutritional status, poor estrus manifestation and improper timing of insemination. The later two are related to the direct result of lack of owner‘s awareness.

The application of reproductive hormones to improve estrus detection resulted in optimal estrus response rate. Though the situation of poor nutrition hindered the dramatic change in pregnancy, it has significantly improved the pregnancy both in the zebu and cross breed animals that were reared under the same management. Although the need for estrus detection has been removed, owners still had the role of bringing the animals within the optimal period for insemination. In this regard, owners experience still had a significant role as clearly shown in their difference of pregnancy success between cows owned by those with good awareness and those without. Dairy owners within the towns were known to principally hold cross breed animals, and had a relatively better experience of both nutritional and breeding management while owners residing in far PAs were found to be unaware of basic estrus signs, were reluctant, and had poor management. Concomitant with this are those linked to the difficulty of procurement of semen and liquid nitrogen, inseminators skill, lack of logistics needed to carry out a smooth AI delivery to far located PAs, repeat breeding and prevalent reproductive health problems. Based on the above highlighted conclusions, the following recommendations are forwarded:

- Creation of awareness on breeding and feeding management to cattle owners through extension programs
- Improving the nutritional conditions of animals selected for dairy production is of paramount importance
- Improving estrus detection methods, subsequent proper timing of insemination and pregnancy diagnosis is required to improve efficiency of the AI program
- Besides the stationed AI service, there need to be a mobile and outreach services to increase the accessibility of AI to the small holders.
- Improving the logistics required for a smooth AI delivery system is important. Field equipment, semen transport and storage facilities, sufficient liquid nitrogen, thaw units, and related insemination facilities have to be availed to conduct proper service.
- Improving the skill of the inseminators through on job training is very important.
- Addressing the prevailing reproductive health problems should be given due veterinary attention.

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8. ANNEXES

Annex I: Data collection format for experimental study

Owner‘s name ______________ District __________PA __________ Sub PA ___________

1. COW IDENTIFICATION AND HEAT INDUCTION DATA

cow ID	Breed	previous AI	color	parity	BCS	milk yield	reproductive problem	herd no.	Last C.date	Estrumate injection(2ml)		Active cop.lutium	
										Date	time	RO	LO
1													
2													

2. TYPE OF ‗HEAT SIGNS OBSERVED

Cow No	Estrus signs exhibited		Bellow	Stand	Mount	Mucus	Turgid uterus	Decrease milk	Insemination service		PD result	
	Date	time							Date	time	+ve	_ve
1												
2												

Annex II: Data Collection Format for Questionnaire Survey from Owners

1. Owner's name ____

District ______________PA _____________Sub PA __________

2. Cow/heife Identification

Breed of the cow: Local ☐ Cross ☐ Blood level _______

Age _________

Parity 1_____ 2_____3_____4_____

Body condition: Scoring 1-3____ 4-5 _____ 6-9 ____

3. Breeding Status of the cow

   Last calving Date ______________

   No. service got ALC ____________

   Last Date of Service _____________

   Type of mating used _____________

4. History of reproductive Problems Encountered

   RFM _____ Abortion ______ Dystocia _____ Prolapses ____Others_____

5. Health status

   Deworming _____ Vaccination _____Others______

6. Rearing System: Free range system______; If tethering, housing and feeding status____

7. Heat Identification

   Experience of the owner using AI service before: Yes _____No _____

   If yes, when present the cow to AI service ___________

   Knowledge of owner in heat identification (Major oestrus Signs) _________

8. Major Heat Signs Observed During Insemination by:

   Owners

   Type of signs ____

   ____

   Date ___________________Time_____________

   Time of calling/presentation of the cow/heifer to the inseminator_______________ (If possible)

   Inseminator

   Time of cow presented/call received_______________

   Time of insemination service given________________

Major types of heat signs observed:

____

____

Personal judgment of heat during insemination: Before time______ on time_____ passed______

Method of thawing _______________

9. Type of Semen used

   Source__________________________

   Bull no.__________ Breed__________ B/d level ______ Straw no. and age______

10. Distance of the sub PA from the station___________

11. Number of animals getting the service per day (max & min no.)___________________

12. Method of pregnancy diagnosis

    Date___________________

    Rectal palpation; Result +ve_____ -ve_____

    Ultrasonography; Result +ve_____ -ve_____

Annex III: Questionnaire used to collect information from Inseminator

1. No of stations in the District _____
2. Source of semen and nitrogen _______
3. Amount of nitrogen and number of straws providing per trip from the source ______________________ Average no. of monthly insemination _______
4. Mobile service______ If yes, availability of motor, fuel, tire and others ____
5. Mobile service approach: By calling____, By supervision ____Shortage of: nitrogen Yes____ No____, Semen Yes_____, No______
6. Data recording______, No. of nitrogen containers ________
7. Level of education ______ Experience ______
8. Method of pregnancy diagnosis after insemination: NRR_____ Rectal PD test _____ No ______

9. How can you check quality of semen? _____________
10. What are major problems in the current AI service?
11. What do you think about the causes of major problems/constraints hindering the AI delivery?
12. Comment alternatives in improving the work of AI service to change the existing situation

Annex IV: A Guide to Body Condition Scoring of Zebu Cattle

Score	Condition	Features
1	L-	Marked emaciation (animal condemned at antemortem examination)
2	L	Transverse process project prominently, neural spines appear sharply
3	L+	Individual dorsal spines are pointed to the touch: hips, pins, tail-head and ribs are prominent
4	M-	Ribs, hips and pins clearly visible. Muscle mass between hooks and pins slightly concave. Slightly more flesh above the transverse process than in L^{+}.
5	M	Ribs usually visible, little fat cover, dorsal spines barely visible
6	M+	Animal smooth and well covered: dorsal spines cannot be seen, but are easily felt
7	F-	Animal smooth and well covered, but fat deposits are not marked. Dorsal spine can be felt with firm pressure, but felt rounded rather than sharp
8	F	Fat cover in critical areas can be easily seen and felt; transverse processes cannot be seen or felt
9	F+	Heavy deposits of fat clearly visible on tail-head, brisket and cod; dorsal spines, ribs and pins fully covered and cannot be felt even with firm pressure

Source: Nicholson and Butherworth, (1986)

Annex V: Pictures of Field Work during Estrus Induction

A. Orientation of owners at the day of injection

B. Animals congregated for heat induction

C. Animals waiting for rectal palpation

D. Hormone used during heat induction and animals restrained for hormone injection

E. Ultrasonic examination of pregnancy in induced cows 25 days post insemination

SIGNED DECLARATION SHEET

The undersigned, declaration that the thesis is my original work and has not been presented for degree in any university and all source of materials used for the thesis have been dully acknowledged.

Name___________________________

Signature________________________

Date of submission_________________

This thesis has been submitted for examination with my approval as University advisor.

Name_________________________	Name_________________________
Signature_______________________	Signature_______________________
Date __________________________	Date __________________________