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# Evaluation of Response to Super-Ovulation, Estrous Synchronization and Embryo Transfer in Local Zebu or Crossbred Dairy Cattle

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*የጥናቱ ዋና ዋና ዓላማ ለፅንስ ምርት የተመረጡ ፅንስ ሰጭ የወተት ላሞችና ጊደሮች ላይ በአማካይ የሚገኘውን የፅንስ ብዛት እንዲሁም ፅንስ ለሚተላለፍባቸው የተመረጡ ፅንስ ተቀባይ እንሰሳት የድራት ግብረ መልሳቸውን እና ፅንስ ከተላለፈ በኋላም የእርግዝና መያዝና መርጋት አቅማቸውን ለመፈተሽ ነው፡፡ የፅንስ መሰብሰቡ ተግባር የፅንስ በገፍ ማኩረቻና ማምረቻ መንገዶችን (ሱፐር ኦቩሌሽን ፕሮቶኮሎችን) በመጠቀም 19 የሆሊስቲን ፊሪዢያን እና የቦረና ዲቃላ የወተት ላሞች ላይ ተከናውኗል፡፡ ለፅንስ ማስተላላፍ ወይም ማዘወር ተግባር 113 የሆሊስቲን ፊሪዢያን እና የቦረና ዲቃላ የወተት ላሞች ላይ ድርያ (የኮርማ ፍላጎት) ማቀናጀት ፕሮቶኮሎችን በመጠቀም የኮርማ ፍላጎት ማቀናጀት ስራ ተከናውኗል፡፡ 34 (24 በማቀዝቀዝ የቆዩና 10 አዲስ የተሰበሰቡ ፅንሶች) ወደ ፅንስ ተቀባይ ላሞች ተላልፏል፡፡ የዚህ ጥናት ውጤት እንደሚያሳየው ፅንስን በገፍ የማምረት ዘዴን በመጠቀም ፅንስ ከተሰበሰበባቸው ከእያናዳንዷ ፅንስ ሰጭ ላሞች ላይ በአማካይ 2.07 ፅንስ የተገኘ ሲሆን የድርያ ግብረ መልሳቸው በሰውነት አቋም ደካማነት ምክንያት ዝቅተኛ ሆኖ ሲመዘገብ የከብቶች ዝርያ እና የወሊድ መጠን (ፓሪቲ) በውጤቱ ላይ የጎላ ተፅዕኖ አለማሳየቱ ተመዝግቧል፡፡ ለፅንስ ዝውውር ለማመቻቸት ሲባል ድርያ (የኮርማ ፍላጎት) ለማቀናጀት ሆርሞን ከተወጉ ላሞች መካከል 46% ቀና ምላሽ ሰጥተው ወደ ፍላጎት መጥተዋል፡፡ በፅንስ ዝውውር ዘዴው አጠቃላይ ያረገዙ ላሞች በአማካይ 20% ሲሆን የላሞች የሰውነት አቋም በውጤቱ ላይ ተፅዕኖ እንዳሳደረ በጉልህ ታይቷል፤ የከብቶቹ ዝርያ፣ የፅንሱ ዓይነት(የፍሪጅ፣ ትኩስ)፣ እና የላሞች የወሊድ መጠን በስራው ላይ የሚስተዋል ተፅዕኖ አላሳዩም፡፡ ለማጠቃለል በዚህ ጥናት ላይ በአማካይ ከእያንዳንዱ ፅንስ ሰጭ ላም የተገኘው የፅንስ ቁጥር አነስተኛ ነው፡፡ እንዲሁም የተቀባይ ላሞች የድርያ ማቀናጀት ምላሽና የእርግዝና መያዝና መርጋት መጠኑም የቀነሰ ሆኖ ተመዝግቧል፡፡ ደካማ የድርያ ማቀናጀት ምላሽ እና የእርግዝና መያዝና መርጋት አነስተኛ መሆን ከደካማ የሰውነት አቋምና ከፍተኛ የለጋ ፅንስ መከሰም ጋር ይያያዛል፡፡*

## Abstract

*An experiment was conducted at Holetta and Debre Zeit Agricultural Research Centers dairy herd in 2011 to evaluate the number of embryos collected per super-ovulated donor cow, estrus rate (ER) and pregnancy rate (PR) of recipient cows/heifers after embryo transfer. For super-ovulation treatment a total of 19 Holstein Friesian (HF) x Boran crossbred cows were selected as embryo donors and treated with super-ovulation hormones. For embryo recipient about 113 females of pure Boran and Holstein Friesian x Boran crossbred cows and heifers synchronized with estrous synchronization hormones. Out of 52 cows and heifers in estrous after estrous synchronization 34 of them used as embryo recipient out of which 23 females received fresh embryos and 11 frozen embryos. All data were analyzed using frequency distribution and Chi-square test. Results from super-ovulation response indicated that out of 19 cows super-ovulated 15 (79%) cows responded to super-ovulation treatment. A total of 31 embryos collected out of which 23 embryos (77%) were transferable and the rest were not suitable for transfer. Mean number of embryo collected per donor was 2.07. Results from recipient estrous synchronization indicated that the overall recipient ER to estrous synchronization was 46% and significantly ($P < 0.05$) influenced by body condition score (BCS) while the effect of synchronization protocols, parity and breed were not significant. The overall recipient PR to embryo transfer was 20% and significantly influenced by BCS ($P < 0.05$). It can be concluded that the number of embryo obtained per super-ovulated donor in present study is low. Moreover recipient PR to embryo transfer was also very low. The poor PR of recipient females to embryo transfer could be attributed to high early embryonic mortality. Further research is required to investigate the possible cause of low number of embryo per donor cows and the poor PR of recipient females to embryo transfer.*

# Introduction

In Ethiopia, despite large cattle population (52 million), favorable climatic conditions and market access, the self-sufficiency in milk production is not yet attained. In recent years, per capita consumption of milk decreased from about 26 liters in mid 1980s to about 16 liters in 2001 (Muriuki and Thorpe, 2003, FAO 2004) which is by far much lower than African and global standard. This is mainly attributed to low number of breeds improved for productivity, lower reproductive performance of both indigenous and crossbred cows. The improvement effort made so far to increase individual or herd level productivity in Ethiopia was fully based on the effort to cross indigenous breeds with highly productive exotic breeds through AI. Nevertheless, the highly productive dairy cattle with exotic blood and their crosses constitute less than 1% of the national cattle population (Zemalem *et al*., 2011). Moreover, despite the three decades crossing breeding exercised, there are no good sources of breeding stock in the country for those who want to go into dairy production business (Zemalem *et al*., 2011). On the other hands, the application of animal reproductive biotechnology can greatly accelerate the speed at which desirable characteristics, for example, better growth rates, or increased milk production can be introduced into animals. While classical breeding to enhance animal traits works well, it takes decades to produce major changes. A recent breakthrough in animal reproduction is the combined application of Artificial insemination (AI), embryo production and transfer and estrous synchronization. Artificial insemination provides an economically viable technique to introduce desired genetics into a herd from male side, whereas synchronization of estrus, ovulation, or both provides a more labor-efficient way to incorporate AI and embryo transfer (ET) into management practices, whereas embryo transfer permits multiplication of desired female animals (Tegegne *et al*., 2004). If these opportunities are pursued they are likely to drive the population into specialized strains of dairy and beef cattle.

# Materials and Methods

## Study area

The study was undertaken at Debre Zeit and Holetta Research Centers, Ethiopia in 2011. Holetta is located in the central highlands of Ethiopia at $9^0$ 3′N latitude and $38^0$ 38′ longitude, 34 km west of Addis Ababa. The area is situated at an altitude of 2400 meters above sea level. It receives an average annual rainfall of 1000 mm. The minimum and maximum ambient temperature during 1974 to 2002 was 6 and $22^0$C respectively. The area receives an average annual rainfall of 800-1000 mm with a minimum and maximum ambient temperature of 10 to 24 $^0$C, respectively.

## Experimental animal selection

The study animals were constituted of indigenous (Boran) and HF x Boran crossbred females. Candidate donor and recipient females were selected on the bases of their previous productive and reproductive performance, physical fitness, docility, health records and reproductive status. All animals were evaluated for animal health and BCS before the start of treatment.

## Donor's super-ovulation

A total of 19 HF x Boran crossbred cows (7 cows from Holetta and 12 cows from Debre Zeit Research Center dairy herd) were treated with super-ovulation protocol. At day zero (start of the experiment) intra vaginally Controlled Drug Release (CIDR) was inserted simultaneously with an injection Estradiol benzoate (EB; 2mg). A decreasing dose system was used for FSH injection (3 ml of FSH "bid" on day 5; 2.5 ml FSH bid on day 6; and 2 ml FSH bid on day 7). CIDR was removed on day 7 while injecting the second shot of FSH with additional injection of $PGF_{2\alpha}$ (5 ml lutalyse). The donor cows were bred twice (in the morning and afternoon) based on the manifestation of standing heat and the second breeding was done 24 h after the first breeding. Embryo flushing was done on 7$^{th}$ day after the last breeding. The ovarian response and number of corpus luteum (CL) were estimated by manual palpation and/or ultrasonography. Number of embryo collected, quality of embryo and fresh transferred embryos were recorded.

## Recipient estrous synchronization

A total of 46 cows and 67 heifers were selected to be used as recipient by manual examination. All animals were checked for any type of abnormalities that affect reproductive performance of the animal and health problems. Aparently healthy cows/heifers were used based on fair or good body condition score (4 and above on 1 to 9 scale). The estrus synchronization experiment consisted of three estrous synchronization protocols, two breeds (Boran and HF x Boran), two BCS classes (females with BCS = 4 and those with BCS greater than or equal to 5), two parity groups; (cows and heifers).

Animals were assigned randomly to one of the three treatments: treatment 1; EB + CIDR + $PGF_{2\alpha}$ and treatment 2; GnRH + CIDR + $PGF_{2\alpha}$. Treatment 3; CIDR + $PGF_{2\alpha}$. Cows and heifers allocated to treatment 1 injected intramuscularly with 2 mg of EB (Ciderol, Genetics, Australia) and a CIDR (1.9 g of progesterone; Pfizer Animal Health, New Zealand) implant was inserted into the vagina. Seven days later, the CIDR was removed and each cow/heifer was injected intramuscularly with 2 mL of $PGF_{2\alpha}$ (Estrumet; Intervet). Cows/heifers allocated to treatment 2 were treated similarly except that GnRH (2 ml; Busereline acetate-Receptal; Intervet¨) was used in place of EB. Cows/heifers allocated to treatment 3 were treated with CIDR insert and seven days later, the CIDR was removed and each cow/heifer was injected intramuscularly with 2 ml of $PGF_{2\alpha}$. After CIDR removal all animals were observed for behavioral estrus

## Detection of estrus

After completion of the synchronization treatment, animals were observed for estrus behavior by trained fulltime (24 hour) observers for 5–7 days and estrous behavior was recorded. During the 7 days after $PGF_{2\alpha}$ injection, treated animals were maintained in a holding yard to increase the accuracy of observations. Onset of estrus was determined when the first two mounts was received within the 4 hours period. The end of estrus was the last mount received, with a mount 4 hours before, and no mounts received during the next 12 hours (White *et al*., 2002).

## Embryo transfer

Out of 52 females responded to estrous synchronization treatments only 34 females used as embryo recipient. A total of 34 embryos (23 fresh and 11 frozen) were transferred to recipient (Boran and HF x Boran cows or heifers at day 7 after recipient observed in estrus. Pregnancy to embryo transfer was determined 40 days after embryo transfer using rectal palpation and ultrasound.

## Statistical analysis

Mean number of embryo per donor cows was determined using descriptive statistics (SAS, 2004). Frequency analysis and chi-square test were used to determine ER of recipient females using estrous rate as outcome variable (if estrous occurred= 1, if not=0) and the class variables used in the model were BCS, parity, synchronization protocol and breed of cow/heifer.

Frequency analysis and chi-square test were used to determine PR of recipient females to embryo transfer using PR as outcome variable (If pregnancy occurred= 1 if not=0) and BCS (4 or 5 and above), parity (cows and heifers), breed (Boan or HF * Boran) and embryo type (fresh or frozen) as class variables.

Location effect was included for all traits in the preliminary analyses of the data and was appeared not significant for all traits. In addition there was small number of observation per location especially for PR to embryo transfer. Therefore, we removed location effect from the model in the final analysis.

# Result and Discussion

## Super-ovulation responses

Results on super-ovulation responses (Table 1) indicated that a total of 19 donors (crossbred cows) were super-ovulated, 15 cows (79%) were responded to super-ovulation treatments. A total of 86 CL were observed ranging from 5 CL per cows to 12 CL per cows with mean of 7.3 CL per cow. A total of 31 embryos were recovered. From those recovered embryos 23 (77%) of them were transferable quality. Mean number of embryo per donor cow was estimate to be 2.07.

Number of embryo recovered in present study was lower than literature reports. In *Bos taurus* breed, using different doses of FSH/LH the number of embryo recovered reported to be from 5.3 to 13.2 (Willmott *et al*., 1990). Tribulo *et al*. (1991) using Brahman (*Bos indicus*) crosses the number of embryo after super-ovulation with FSH ranged from 4.6 to 8.5. Using FSH, Krininger *et al.* (2003) also reported 9.3 embryos for Brahman and 10.2 embryos for Holstein Frisian females. The relatively lower number of embryo found in present study compared to literature report could be related to physiological difference in reproductive behavior, environmental effects or both. Some studies suggested breed differences in the physiology and the reproductive behavior (Baruselli *et al*., 2006) can affect the efficiency of super-ovulation programs. Response to super-ovulation hormones could also be affected by age, breed of donor and individual animal physiological condition at the time of treatment (Mapletoft *et al.*, 2002; Baruselli *et al.*, 2003). Difference between breeds in response to super-ovulation is reported by different authors. In Beef cattle breed the number of embryo collected and number of transferable embryo found to be 2.8 and 2.1 respectively (Gonzale *et al.*, 1994). However, **u**nder Brazilian condition small variation was observed for zebu breed with 6.7 embryos for Nelore breed (Peixto et al., 2006; Hanson et al., 2004), 7.3 embryos for Brahman, 4.1 for Gir and 5.7 for Guzera (Peixto et al., 2006). A response of 5 to 6 transferable embryo is well accepted in several European breed (Castro Neto *et al.*, 2005). The lower super-ovulation responses in present study compared to most of the study could also be related to time of initiation of super-ovulation treatment, as super-ovulation treatment in present study was initiated without considering follicular development stage. Some studies suggested that the importance of initiating gonadotropin treatments at the time of follicular wave emergence (Bo *et al.*, 1995; Son *et al.*, 2007). Inability to start super-ovulation treatments at the optimal time of follicular development is the major causes of higher variability in embryo production (Baruselli *et al.*, 2003; Martins *et al.*, 2005; Baruselli *et al.*, 2006).

Table 1. Donors response to super ovulation treatment

| Herd | Donor treated | Donor Super ovulated (no/%) | Embryo recovered | Mean number of embryos recovered/donor |
|---|---|---|---|---|
| Holetta | 7 | 6 (86) | 15 | 2.5 |
| Debre Zeit | 12 | 9(75) | 16 | 1.78 |
| Total | 19 | 15(79) | 31 | 2.07 |

## Estrus responses of recipient cows and heifers

The estrus response of females synchronized with estrous synchronization protocols are presented in Table 2. The proportion of Boran and Boran x HF crosses that responded to synchronization treatment was 48.72 and 44.59% respectively ($P > 0.05$). There was no significance difference among estrous synchronization protocols. The effect of parity on ER was not significant however ER was higher for cows compared to heifers. Contrary to our finding, difference between cows and heifers in estrous behavioral signs was reported (million et al., 2011). The lack significance difference in ER between cows and heifers could be related to small number of observations in present study. Estrus rate was significantly ($P < 0.05$) higher for females with BCS equal or greater than 5 compared to females with BCS equal to 4. The significantly higher ER in females with BCS equal or greater than 5 compared to those with BCS equal to 4 reflects the role of nutrition in reproduction. It is a well-established fact that body condition is an indicative of nutritional status of animal, good body condition is an indicative of better nutritional status while poor body condition is usually related to poor nutrition status (DeRouen *et al.*, 1994).

Table 2. Estrus rate of Boran, and Boran × HF crossbred dairy cattle in response to estrus synchronization protocol

| | | No treated | No in estrus | Estrus rate (%) | Chisq | P>Chis |
|---|---|---|---|---|---|---|
| Breed | | | | | 0.17 | 0.6759 |
| | Boran | 39 | 19 | 48.72 | | |
| | Cross | 74 | 33 | 44.59 | | |
| | | 113 | 52 | 46.02 | | |
| BCS | | | | | 12.27 | 0.0008 |
| | 4 | 83 | 30 | 24.10 | | |
| | 5 and above | 30 | 22 | 73.33 | | |
| Parity | | | | | 1.01 | 0.6026 |
| | Cow | 46 | 33 | 71.74 | | |
| | Heifer | 67 | 29 | 43.28 | | |
| Treatment | | | | | 1.92 | 0.38 |
| | EB+CIDR+ $PGF_{2\alpha}$ | 26 | 15 | 57.69 | | |
| | GnRH+CIDR+ $PGF_{2\alpha}$ | 55 | 24 | 43.64 | | |
| | CIDR+ $PGF_{2\alpha}$ | 32 | 13 | 40.63 | | |

## Recipient pregnancy rate after embryo transfer

Results on recipient PR per embryo transfer are presented in Table 3. PR was significantly (P < 0.05) influenced by BCS, while the effect of breed, embryo type and parity were not significant. Although the difference was not significant, females received frozen embryo had higher PR (36.36%; P > 0.05) compared to those received fresh embryo (17.04%). PR was slightly higher for Boran x HF crosses compared to pure Boran (27.27% versus 17.39%: P > 0.05). Although the difference was not significant heifers had higher PR (23.08%) than cows (12.50%). Mean PR was significantly higher (40%; P < 0.05) for females with BCS ≥ 5 compared to females with BCS equal to 4 (5.26%).

Table 3. Pregnancy rate of females after embryo transfer by sources of variations

| | Number of females received embryo | Pregnant | | Chisq | P>Chisq |
|---|---|---|---|---|---|
| | | Number | % | | |
| Overall | 34 | 7 | 20.59 | | |
| Embryo type | | | | 2.48 | 0.1157 |
| Fresh | 23 | 3 | 13.04 | | |
| Frozen | 11 | 4 | 36.36 | | |
| Breed | | | | 0.44 | 0.5050 |
| Boran | 23 | 4 | 17.39 | | |
| Cross | 11 | 3 | 27.27 | | |
| Parity group | | | | 0.4186 | 0.5176 |
| Cow | 8 | 1 | 12.50 | | |
| Heifers | 26 | 6 | 23.08 | | |
| BCS | | | | 11.16 | 0.0008 |
| 4 | 19 | 1 | 5.26 | | |
| 5 and above | 15 | 6 | 40.00 | | |

Pregnancy rate per embryo transfer observed in present study is lower than the PR of 59.7% for Nellore and 68.3% for Gyr embryos that was reported by (Peixoto *et al.*, 2007).

The poor PR observed per embryo transfer in present study was attributed to early embryonic mortality six embryo mortalities out of 13 established pregnancies (46%) were encountered at about 45-60 days post transfer. Unfortunately, the precise cause(s) of this embryonic loss are not clear. However, the lower PR observed in animals with BCS equal to 4 is related to poor nutrition or energy status of recipient females. Investigations on postpartum reproduction in cattle indicate that BCS is a useful indicator of energy status and rebreeding potential (DeRouen *et al*., 1994). Evidence indicated that poor nutrition is a recognized cause of reduced fertility in cattle grazing in subtropical/ tropical areas (Bó *et al*., 2003). Poor feed availability during the dry season could be contributed for poor embryo survival, since this experiment was conducted in the middle of the dry season when forage supply was inadequate and poor in quality.

Cattle embryos have an increased requirement for glucose from around the 8 -16 cell stage of development and particularly at about day 7, at the start of blastocyst formation. Furthermore, glucose uptake by cattle embryos at this time is positively correlated with their capacity to develop further (Diskin *et al* 2001). Low glucose concentrations in a critical period post-AI as a result of a sudden reduction in energy intake may, at least in part, be responsible for the reduction in embryo survival in present study (Diskin *et al*., 2001). Differences related to physiological and immunological aspects of recipients and donors were not considered (Hill *et al*., 2002; Hansen *et al*., 2004) in this study and could be contributed to poor embryo survival and PR.

## Conclusion and Recommendation

It can be concluded that number of embryo collected per super-ovulated donor cows, recipient ER and PR to embryo transfer were low compared to literature results. The sample size in the present study is very small to give conclusive remark. However, the poor super-ovulation response is related to physiological condition of animal at the time of initiation of super-ovulation treatment. The poor ER of recipient females in present study is attributed to poor body condition of animal at the time of initiation of synchronization treatments. The poor recipient PR is attributed to poor BCS and high embryo mortality. There is a need to improve feeding management of animals through providing balanced feed especially energy and protein content and maintain body condition between 5-7 at the time of estrous synchronization treatment and embryo transfer. Moreover super-ovulation treatment needs to be initiated at the optimal time of follicular development stage. Since the present study is based on small number of sample size, future research is required with more number of animals to investigate the poor super-ovulation responses and poor PR of recipient female to embryo transfer.

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