Comparative study on efficiency of sexed semen and conventional semen on in vivo produced bovine embryo quality and quantity of Boran and Holstein-Boran cross bred in Bishoftu, Ethiopia

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The study was conducted from December 2019 to May 2020 to evaluate the efficiency of local conventional semen and imported sexed semen on in vivo produced bovine embryo quality in Boran and HB cross breed donor cows in Bishoftu, Ethiopia. In this study, it was hypothesized that the breed of donor cows and types of semen can influence the quality of in vivo produced embryo as well as the number of transferable embryos. Randomized experimental design was employed, and a total of 20 donor cows (10 Boran and 10H* B cross) were superovulated and flushed excluding non-responsive donor cows. Donor cows were randomly assigned to two groups: Group-1- five donors inseminated with sexed semen and group-2- five other cows inseminated with conventional semen from each breed. The results of study showed that the embryo recovery rate was 68% in H* B crossbreds and 53% in Boran donor cows. A total of 121 embryos were produced of which, 60 were transferable embryos (36 from H* B crossbreds and 24 from Boran), 39 UFOs (31 from H* B crossbreds and 8 from Boran) and 22 degenerated embryos (12 from HB cross and 10 from Boran). The results of this study showed that the number of transferable embryos produced from donors inseminated with sexed semen and conventional semen was nearly similar. However, the number of defective embryos tended to be higher with sexed semen and the number of unfertilized oocytes is significantly (p<0.05) higher in crossbred cows. This finding suggests that farmer can use sexed semen to get sex specific dairy calves in both cattle genotypes.

Key words: Breed, donor cows, superovulation.

INTRODUCTION

Ethiopia holds the largest cattle population in Africa which is estimated to be about 59.5 million heads of cattle. The indigenous breeds including Boran cattle account for 98.20%, while the hybrids and pure exotic breeds represent 1.62 and 0.18% respectively (CSA, 2017). However, its contribution to the overall production has
shown low productivity a due to their low genetic potential 
and poor technical knowledge on the part of dairy owners 
(Yehalaw et al., 2018). The low productivity of the 
country’s livestock production system has been 
prssumed to be due to shortage of crossbreed dairy cows 
and high number of indigenous breeds of low genetic 
potential for milk production (FAO, 2007). However, 
Ethiopia has large potential for dairy production 
development due to the country’s favorable climate in the 
highlands which supports use of improved, high yielding 
dairy breeds and offers a relatively disease-free 
environment for livestock development (Ahmed et al., 
2004). Following artificial insemination (Al), oestrus 
synchronization and mass artificial insemination (OSMAI) 
was operated as a second reproductive technology. 
Embryo transfer (ET) was also started in Debre Zeit 
research center but the technology has not become 
expanded due to different constraints (Tegegne et al., 
2016; Tamrat et al., 2018). With the OSMAI technology, 
one genetically superior crossbred cow can produce up 
to 32 embryos per year compared to the conventional 
method of breeding where the farmer has to wait for 
twelve months or more for a single calf that could be 
either male or female (Muchemi, 2011).

Following superovulation protocol (Larson et al., 2010), 
the number of viable embryos collected from a donor cow 
in one embryo collection session is an average of six, but 
varies considerably among different donor cows, with 
approximately 15% of embryo collections resulting in 
non-transferable embryos and a small number of donors 
producing 20-50 embryos (Baruselli et al., 2006). The 
unpredictable individual variation in the superovulation 
response is a major limiting factor determining the 
efficiency of MOET in breeding programs dependent on 
a number of factors related to breed donor, environment 
and management (Marja, 2017). The embryo transfer 
technology has been applied in wide area of the world 
because it increases the number of offspring that can be 
obtained from donor cow with high genetic value 
(Baruselli et al., 2006; Bó et al., 2006). Again, the use of 
sexed semen has created an opportunity of getting 
embryos with a specific gender that would economically 
benefit beef and dairy industries worldwide (Sartori et al., 
2004).

Studies on the efficiency of Al with sexed semen and 
conventional semen following super ovulation on in vivo 
production of embryo quality and quantity in different 
zebu breeds are limited in Ethiopia. Therefore, this study 
was conducted to evaluate the effect of conventional and 
sexed semen on quality of in vivo produced embryos as 
well as breed effect in response to types of semen in 
Boran and their Friesian crosses.

MATERIALS AND METHODS

Experimental Animals

A total of 20 cycling donor cows (10 Boran and 10 crossbreds) were 

included in this study after clinical and gynaecological examination 
of the reproductive tracts for any abnormalities and to determine the 
reproductive status of individual animals using rectal palpation and 
ultrasonography (Tamrat et al., 2018). Only cycling (with active CL) 
and apparently healthy donor cows without any reproductive 
disorder were selected as candidates for superovulatory treatment. 
All cows were managed under similar housing, feeding and 
health management and fed teff straw and grass hay as a basal 
diet that was supplemented with commercially prepared concentrate 
feed (mixed from wheat bran, wheat middling, corn, Noug cakes, 
and mineral salts) and green fodder (fresh grass, alfalfa, elephant 
grass). Feeding was based on the level of production, and stage of 
reproduction, while water was given daily ad libitum. Animals were 
dewormed with broad-spectrum anthelmintic and vaccinated 
annually against anthrax, black leg, FMD, pasteurellosis, CBPP and 
LSD.

Ethical considerations in this study were taken seriously. None of 
the procedures involve undue stress to the experimental animals 
and were handled humanely and hence it did not inflict any harm or 
unnecessary discomfort to the animals. All activities were carried out in accordance with the ethical 
guideline of the Addis Ababa University College of Veterinary 
Medicine and Agriculture after receiving the ethical approval under 
(Ref. No: VM/ERC/34/03/12/2020).

Study design

Randomized experimental design was employed to study the 
effects of breed and type of semen on the quality of produced 
bovine embryos. Experimental animals were grouped based on 
breed (Boran and crossbreds) and randomly assigned to the types 
of semen; local conventional semen and imported sexed semen with 20 × 10⁶ sperm cells per ml. Local 
semen was produced from Boran and crossbred cows. Both 
local and imported semen were inseminated twice per day with 
12 h interval post standing heat (Fig 1).

Donor superovulatory procedure

A total of 20 donor Cows (10 Boran and 10 crossbreds) with active 
CL were assigned in this experiment. On day 0, CIDR 
(Progestrone 1.38 g, Hamilton, New Zealand) were inserted in 
every cow for seven days. On Day 4, cows were assigned to two 
different doses of FSH (Plusei®, Spain) treatment, regimen 250 IU 
for Boran and 700 IU for crossbreds. FSH was administered 
intramuscularly on Day 4 and CIDR was withdrawn on Day 7 during the last injection of FSH 
(Figure 1).

Heat detection and AI of donor cows

After CIDR withdrawal, heat signs were closely observed and heat 
detector (ESTROTECT™ #U.S.pat. #6,467,430) was applied to 
control those animals with short duration of oestrous. Before 
imsemination, one straw of semen from both types of semen (sexed 
and conventional) was thawed and checked for sperm motility. All 
Cows were inseminated twice with 12 h interval post standing heat 
based on the observed heat sign to meet the ovulation time with 
post sperm capacitation.
Table 1. Doses of FSH (IU) used for superovulation treatment for both breed of donor cows.

| Total dose (IU) | Breed          | Dose per day (IU) |
|----------------|----------------|-------------------|
|                |                | Day 1  | Day 2  | Day 3  | Day 4  |
| 250            | Boran          | 100/2* | 75/2*  | 50/2*  | 25/2*  |
| 700            | B*H cross      | 200/2* | 150/2* | 100/2* |

*: indicate given dose administered twice per day at AM &PM.

Figure 1. Superovulation and embryo recovery protocol.
Source: Tamrat et al. (2018).

Response evaluation and embryo collection

Superovulatory response was evaluated using a real-time B-mode ultrasound with 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy) by counting a total number of CL and unovulated follicles present on the ovaries prior to embryo flushing on day 7. The donors were injected 3 to 5 ml of lidocaine epidurally before inserting collection catheter. The embryos were collected using non-surgical technique of embryo flushing with 1000ml of commercial flushing medium (ViGRO™, Bioniche Animal Health, USA) and two-way Foley catheter (18 Fr 650 mm length; MOFA®, Canada).

Evaluation and grading of embryos

After flushing, the medium within an embryo filter was transferred to searching dish for searching embryos/UFOs under a stereomicroscope (Motis SMZ 140/143®, Roanoke, USA). Collected embryos/UFOs were transferred to four well dishes containing holding medium. The embryos were graded based on their developmental stage (from stage 1 = one cell to stage 9 = expanded hatched blastocyst) and quality (from quality 1 = excellent to quality 4 = degenerate) according to the manual of International Embryo Transfer Society (IETS, 2013). Depending on the IETS guidelines collected embryos were categorized as transferable embryos (those in 4-8 developmental stages and a quality grade of 1 and 2), degenerate and UFO.

Statistical analysis

The data were analyzed using STATA version 13 (Copyright 1985-2013 Stata Corp LP, Texas, USA) and P value was calculated using ANOVA to find any significant relationship. Statistical significance of the study considered P value less than 0.05 as significant and P value greater than 0.05 as non-significant.

RESULTS

Superovulatory response

An average of 8.7 and 11.1 of CL, 3.1 and 8 of unovulated follicles were counted from Boran and H*B cross donor cows respectively. A total of 121 recovered embryos/UFOs (75 from cross and 46 from Boran) were evaluated and classified. The number of CL counted from both breeds were not significantly (p > 0.05) different but the number of unovulated follicles were different (p<0.02). Over all, effects of breed on superovulatory response were summarized in Table 2 and maximum number of embryos/oocytes collected from one donor was shown in Figure 4.

Effects of breed on embryo yield and quality

There were no significant (p > 0.05) effect of breed on the number of transferable embryos and degenerated embryos but the number of unfertilized oocytes (Figure 3) across the two breed of donor cows was significantly
Table 2. A mean of superovulatory response in Boran and crossbreed dairy cows.

| Breed         | Response variable | No. | Mean | Std. Dev | Min | Max | F-value | P-value |
|---------------|-------------------|-----|------|----------|-----|-----|---------|---------|
| Boran         | No CL             | 10  | 9.6  | 4.2739   | 4   | 18  |         | 0.51    | 0.4855  |
| H-B cross     |                   | 10  | 11.1 | 5.1088   | 3   | 18  |         |         |         |
| Total         |                   | 20  | 10.35| 4.6484   | 3   | 18  | 0.51    | 0.4855  |
| Boran         | No O              | 10  | 2.7  | 2.6267   | 0   | 7   |         |         |         |
| H-B cross     |                   | 10  | 7.9  | 4.8177   | 1   | 16  |         |         |         |
| Total         |                   | 20  | 5.3  | 4.6237   | 1   | 16  | 8.98    | 0.0077  |
| Boran         | No E/O            | 10  | 4.6  | 4.5995   | 1   | 16  |         |         |         |
| H-B cross     |                   | 10  | 7.5  | 4.7199   | 1   | 16  |         |         |         |
| Total         |                   | 20  | 6.05 | 4.7735   | 1   | 16  | 1.94    | 0.1810  |

Ng CL= Number of corpus Luteum counted, Ng O= number of unovulated follicles, Ng E/O= number of recovered embryos/oocytes, H-B= Holstein Frisian Boran crossbred.

Table 3. Mean of transferable, unfertilized and degenerated embryos.

| Breed         | Response variable | No. | Mean | Std. dev | Min | Max | F-value | P-value |
|---------------|-------------------|-----|------|----------|-----|-----|---------|---------|
| Boran         | TE                | 10  | 2.4  | 3.6270   | 0   | 12  | 0.4575  |         |        |
| H-B           |                   | 10  | 3.6  | 3.4383   | 0   | 9   |         |         | 0.0470  |
| Total         |                   | 20  | 3    | 3.4943   | 0   | 12  | 0.58    |         |        |
| Boran         | UFOs              | 10  | 0.9  | 0.8755   | 0   | 2   |         |         | 0.6432  |
| H-B           |                   | 10  | 3.1  | 3.1428   | 1   | 11  |         |         |        |
| Total         |                   | 20  | 2    | 2.5131   | 0   | 11  | 4.55    |         |        |
| Boran         | DE                | 10  | 1.2  | 1.0327   | 0   | 3   |         |         |        |
| H-B           |                   | 10  | 0.9  | 1.7288   | 0   | 5   |         |         |        |
| Total         |                   | 20  | 1.05 | 1.3945   | 0   | 5   | 0.22    |         |        |

Legend: TE= number of transferable embryos, UFOs= number of unfertilized oocytes, DE= number of degenerated embryos, H-B= Holstein Frisian Boran crossbred.

(p ≤ 0.05) different, being higher in HB crosses. The summary of total collected embryos/oocytes is shown in Table 3.

Effects of types of semen on embryo quality

In this study there was no statistically significant (p > 0.05) difference between two types of semen used in producing transferable and non-viable embryos, even though the two types of semen were different in sperm cell concentration in addition to the sorting effect on sexed semen. However, there was a tendency (p<0.1) of sexed semen to produce higher number of defective embryos. The details of semen effect on embryo quality are shown in Table 4, and different stages and quality of embryo shown in Figures 1 and 2.

DISCUSSION

The current study was performed with the objectives of evaluating the effect of convectional and sexed semen on quality and quantity of in vivo produced embryos from two different breeds of donor cows. The superovulatory response in terms of average number of CL counted the mean number and the range of embryos/oocytes obtained from Boran and their Friesian crosses was similar with previous findings (Hasler, 2010). The variability between animals in ovulatory response to FSH-induced superovulation is mainly due to the differences in ovarian activity at the time of treatment (Rico et al., 2009). In this result, the breed variation was identified and there was a significant difference observed as high number of unovulated follicles counted in H-B crossbreds of donor cows as compared with Boran donors in this experiment.

The use of sex-sorted semen in embryo production by superovulation of donor cow is an important tool for genetic improvement in dairy breeds, consequently increasing milk production. The result of this experiment showed that there was no significant (p > 0.05) difference between the two types of semen on produced embryo quality. However, several previous studies have shown that the use of sexed semen in superovulated donor
Table 4. Mean of different embryo quality and developmental stage across the semen type.

| Typ. semen | Response variable | N  | Mean  | Std.dev | Min | Max | F-value | P-value |
|------------|-------------------|----|-------|---------|-----|-----|---------|---------|
| Convectional | TE                | 10 | 3     | 3.1972  | 0   | 9   |         | 1.0000  |
| Sexed      |                   | 10 | 3     | 3.9440  | 0   | 12  |         |         |
| Total      |                   | 20 | 3     | 3.4943  | 0   | 12  |         | 0.00    |
| Convectional | UFOs              | 10 | 2.1   | 3.2472  | 0   | 11  |         | 0.8643  |
| Sexed      |                   | 10 | 1.9   | 1.6633  | 0   | 5   |         |         |
| Total      |                   | 20 | 2     | 2.5131  | 0   | 11  |         | 0.03    |
| Convectional | DE                | 10 | 0.5   | 0.9718  | 0   | 3   |         | 0.0768  |
| Sexed      |                   | 10 | 1.6   | 1.5776  | 0   | 5   |         |         |
| Total      |                   | 20 | 1.05  | 1.3945  | 0   | 5   |         | 3.52    |
| Convectional | Morula            | 10 | 2.2   | 2.8205  | 0   | 9   |         | 0.7461  |
| Sexed      |                   | 10 | 1.8   | 2.6161  | 0   | 8   |         |         |
| Total      |                   | 20 | 2     | 2.6556  | 0   | 9   |         | 0.11    |
| Convectional | Blasto            | 10 | 0.5   | 0.7071  | 0   | 2   |         | 0.1748  |
| Sexed      |                   | 10 | 1.2   | 1.3984  | 0   | 4   |         |         |
| Total      |                   | 20 | 0.85  | 1.1367  | 0   | 4   |         | 2.00    |
| Convectional | Q1                | 10 | 1.9   | 2.2335  | 0   | 6   |         | 0.3431  |
| Sexed      |                   | 10 | 1     | 1.8856  | 0   | 6   |         |         |
| Total      |                   | 20 | 1.45  | 1.1367  | 0   | 6   |         | 0.95    |
| Convectional | Q2                | 10 | 1.1   | 1.4491  | 0   | 3   |         | 0.3104  |
| Sexed      |                   | 10 | 2     | 2.3094  | 0   | 6   |         |         |
| Total      |                   | 20 | 1.55  | 1.9324  | 0   | 6   |         | 1.09    |

TE= number of transferable embryos, UFOs= number of unfertilized oocytes, DE= number of degenerated embryos, Q1= quality grade one, Q2= quality grade two.

Figure 2: 1=Q1S5, 2=Q1S4, 3=Q2S4 and 4=Degenerated embryos.
cow’s results lower fertilization rate than when using non-sorted semen (Larson et al., 2007, 2010; Soares et al., 2011; Kaimio et al., 2013). As Schenk et al. (2009) reported, the lower fertilization rate could be attributed to the low sperm concentration and damage caused by the sorting process followed by freezing (dilution, laser exposure, process speed and centrifugation).

In the present study, the number of transferable embryos recovered after inseminating donor cows with sexed semen and convensional semen was not significantly (p > 0.05) different. This is contrary to those reported by Peippo et al. (2009) and Larson et al. (2010)
that smaller proportion of transferable embryo were recovered with sex-sorted semen compared to the conventional one. However, number of transferable embryos obtained from sexed semen was higher in cross breed donor cows. This may show that the sexed semen used in current study is of good quality and the time of insemination was later as compared to that of conventional semen. In addition, insemination with sex-sorted sperm should be conducted closer to the time of ovulation because sex-sorted sperm has a reduced life span in the female reproductive tract, which is due to possible precapacitation of the sex-sorted spermatozoa (Maxwell et al., 2004; Peippo et al., 2009).

In the current study, the number of unfertilized oocytes and number of degenerated embryos recovered from both types of semen (sexed and conventional) were not significantly (p > 0.05) different. However, the number of degenerated embryos tended to be higher (p<0.1) with sexed semen. There was no significant difference in the number of quality grade 1 but the number of quality grade 2 was higher with sexed semen. Some studies report a decrease in the number of grade1 embryos when the donors were inseminated with sexed semen; whereas others have not found any significant differences. However, Peippo et al. (2009) report that there was no effect of sexed semen on the number of grade 1 embryos among transferable embryos in superovulated donors inseminated with sexed semen. The mean number of transferable embryos that recovered from cow inseminated with sexed semen was 3 which is in the range of previous findings of 2.1 and 3.8 transferable embryos per recovery reported by Larson et al. (2010) and Peippo et al. (2009) respectively.

Conclusion

The study shows that the number of transferable embryos produced from donors inseminated with sexed semen and conventional semen was nearly similar. However, high number of defective embryos tended to be higher with sexed semen and the number of unfertilized oocytes is higher in crossbred cows. This study suggests that farmers can use sexed semen to get sex specific dairy calves in both breeds of cows.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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