Vol. 12(1), pp. 9-16, January-March 2021
DOI: 10.5897/IJLP2020.0751
Article Number: 2BCC9CA65914
ISSN 2141-2448

http://www.academicjournals.org/IJLP

ACADEMIC JOURNALS

International Journal of Livestock Production

Full Length Research Paper

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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Received 3 November, 2020; Accepted 6 January, 2021

The study was conducted from December 2019 to May 2020 to evaluate the efficiency of local convectional 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 convectional 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 convectional 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

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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 presumed 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 (AI), oestrous 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 AI with sexed semen and convectional 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 convectional 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- libtum*. Animals were dewormed with broad-spectrum anthelmintic and vaccinated annually against anthrax, black leg, FMD, pasteurollosis, CBPP and LSD.

Ethical considerations in this study were taken seriously. None of the procedures involve undue stress to the experimental animals and animal handling has been with humane approach 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 convectional semen with 30 x 10^6sperm cells and imported sexed semen with 2 × 10^6sperm cells. Before the start of the experiment, the ovaries of all animals were evaluated using a real-time B-mode ultrasound with 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy) and rectal palpation to check the status of CL and nature of the cervix. Animals with active CL and with medium or large, straight or not curved cervix were selected as candidate for the super ovulation treatment as kinked cervix difficult to pass.

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 (Progesterone 1.38 g, Hamilton, New Zealand) were inserted in both breed for seven days. On Day 4, cows were assigned to two different doses of FSH (Pluset®, Spain) treatment, regimen 250 IU for Boran and 700 IU for crossbreds. FSH was administered through intramuscular injection for four consecutive days in a decreasing dose regimen (Table 1) and twice per day with 12 h interval. On Day 6PGF2α (Estrumate®, France) was injected IM 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^{TM} #U.S.pat. #6,467,430) was applied to control those animals with short duration of oestrous. Before insemination, one straw of semen from both types of semen (sexed and convectional) was thawed and checked for sperm motility. All Cows were inseminated twice with12 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	250/2*	200/2*	150/2*	100/2*

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

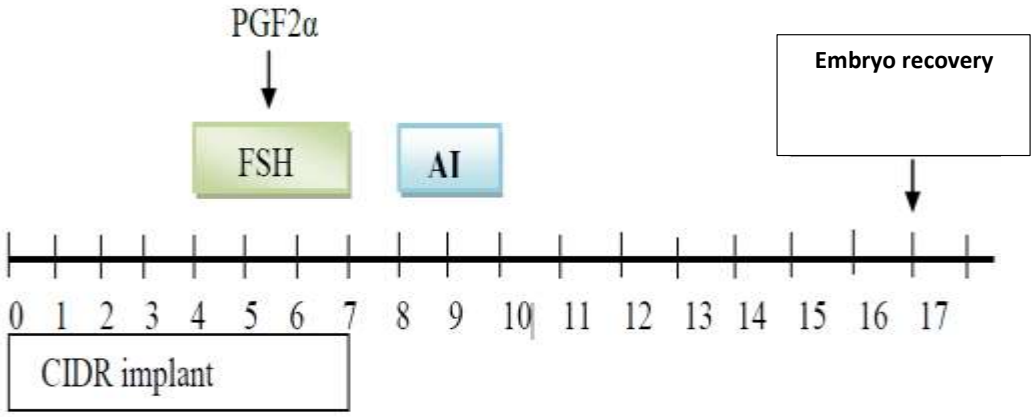

Figure 1. Superovulation and embryo recovery protocol.\
Source: Tamrat et al. (2018).

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		
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	0	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

No CL= Number of corpus Luteum counted, No O= number of unovulated follicles, No 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		
H-B		10	3.6	3.4383	0	9		0.4575
Total		20	3	3.4943	0	12	0.58	
Boran	UFOs	10	0.9	0.8755	0	2		
H-B		10	3.1	3.1428	1	11		**0.0470**
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		0.6432
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.

($p \leq 0.05$) 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		
Sexed		10	3	3.9440	0	12		1.0000
Total		20	3	3.4943	0	12	0.00	
Convectional	UFOs	10	2.1	3.2472	0	11		
Sexed		10	1.9	1.6633	0	5		0.8643
Total		20	2	2.5131	0	11	0.03	
Convectional	DE	10	0.5	0.9718	0	3		
Sexed		10	1.6	1.5776	0	5		0.0768
Total		20	1.05	1.3945	0	5	3.52	
Convectional	Morula	10	2.2	2.8205	0	9		
Sexed		10	1.8	2.6161	0	8		0.7461
Total		20	2	2.6556	0	9	0.11	
Convectional	Blasto	10	0.5	0.7071	0	2		
Sexed		10	1.2	1.3984	0	4		0.1748
Total		20	0.85	1.1367	0	4	2.00	
Convectional	Q1	10	1.9	2.2335	0	6		
Sexed		10	1	1.8856	0	6		0.3431
Total		20	1.45	2.0641	0	6	0.95	
Convectional	Q2	10	1.1	1.4491	0	3		
Sexed		10	2	2.3094	0	6		0.3104
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.

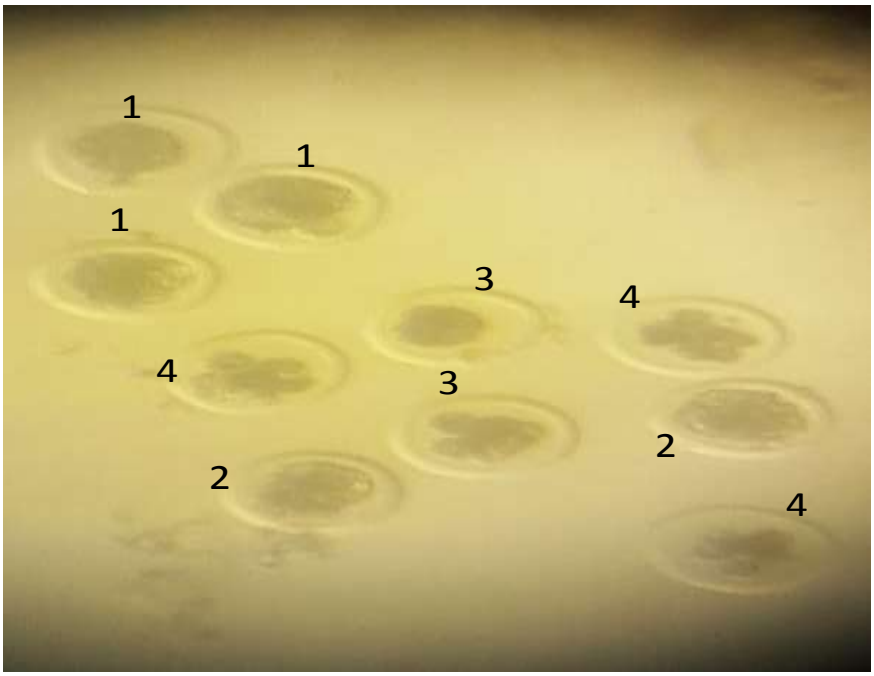

Figure 2. 1=Q1S5, 2=Q1S4, 3=Q2S4 and 4=Degenerated embryos.

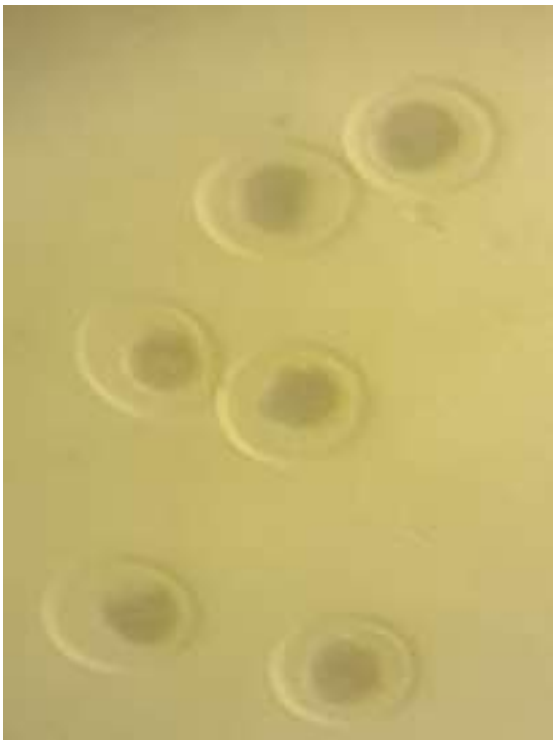

Figure 3. UFO= unfertilized oocytes, Q=quality, S=stage.

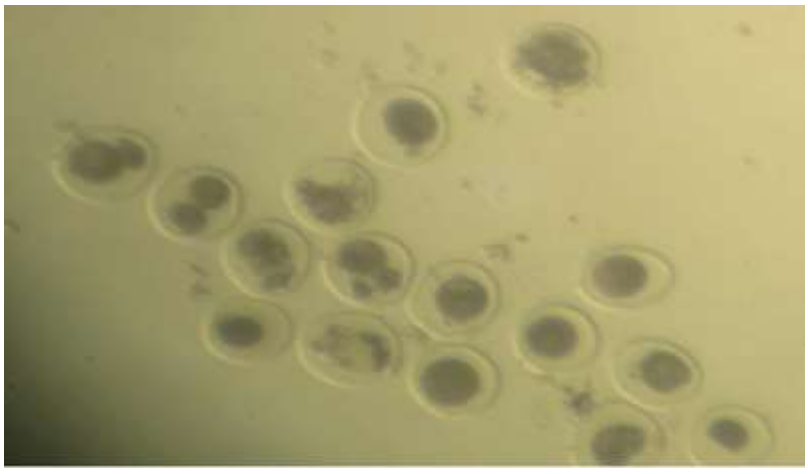

Figure 4. Maximum number of embryos/oocytes collected from one potential donor in both breeds out of all selected donors.

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 convectional 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 convectional 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 convectional) 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 grade1 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 convectional 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.

ACKNOWLEDGEMENTS

The authors appreciate the staff of Animal Biotechnology Laboratory members for their assistance during this work. This study received financial support from the Ethiopian Institute of Agricultural Research.

REFERENCES

Ahmed MAM, Ehui S, Assefa Y (2004). Dairy development in Ethiopia. EPTD Discussion paper No. 123. International Food Policy Research Institute. Washington, USA.

Baruselli PS, Sa Filho MF, Martins CM, Nasser LF, Nogueira MF, Barros CM, Bo GA (2006). Superovulation and embryo transfer in Bos indicus cattle. Theriogenology 65:77-88.

Bó GA, Baruselli PS, Chesta PM, Martins CM (2006). The timing of ovulation and insemination schedules in superstimulated cattle. Theriogenology 65(1):89-101.

Central Statistical Agency (CSA) (2017) Agricultural Sample Survey, 2016/17 (2009E.C.), Volume II: Report on Livestock and livestock characteristics (Private peasant holdings). Statistical Bulletin 585. Addis Ababa, Ethopia: Federal Democratic Republic of Ethiopia; 2017.

Food and Agriculture Organization of the United Nations (FAO) (2007). FAOSTAT 2007.

Hasler JF (2010). Bovine embryo transfer: are efficiencies improving? Applied Reproductive Strategies Conference Proceedings August 5th & 6th, Nashville, TN, pp. 265-282.

Kaimio I, Mikkola M, Lindeberg H, Heikkinen J, Hasler JF, Taponen J (2013). Embryo production with sex-sorted semen in superovulated dairy heifers and cows. Theriogenology 80:950-954.

Larson JE, Lamb GC, Funnell BJ, Bird S, Martins A, Rodgers JC (2010). Embryo production in superovulated Angus cows inseminated four times with sexed-sorted or conventional, frozen-thawed semen. Theriogenology 73(5):698-703.

Larson SF, Butler WR, Currie WB (2007). Pregnancy rates in lactating dairy cattle following supplementation of progesterone after artificial insemination. Animal Reproduction Science 102:172-179.

Marja M (2017). Superovulation and embryo transfer in dairy cattle – effect of management factors with emphasis on sex-sorted semen. Academic dissertation, faculty of veterinary medicine University of Helsinki, Finland.

Maxwell WMC, Evans G, Hollinshead FK, Bathgate R, De Graaf SP, Eriksson BM, Gillan L, Morton KM, O'Brien JK (2004). Integration of sperm sexing technology into the ART toolbox. Animal Reproduction Science 82:79-95.

Muchemi J (2011). New embryo transfer technology to boost dairy farmers' fortune, Sunday Nation, Kenya pp. 26-27.

Peippo J, Vartia K, Kananen-Anttila K, Räty M, Korhonen K, Hurme T, (2009). Embryo production from superovulated Holstein-Friesian dairy heifers and cows after insemination with frozen-thawed sex-sorted X spermatozoa or unsorted semen. Animal Reproduction Science 111:80-92.

Rico C, Fabre S, Médigue C, Di Clemente N, Clément F, Bontoux M, Touzé JL, Dupont M, Briant E, Rémy B, Beckers JF, Monniaux D (2009). Anti-müllerian hormone is an endocrine marker of ovarian gonadotropin-responsive follicles and can help to predict superovulatory responses in the cow. Biology of Reproduction *80*(1):50-59.

Sartori R, Souza AH, Guenther JN, Caraviello DZ, Geiger LN, Schenk JL (2004). Fertilization rate and embryo quality in superovulated Holstein heifers artificially inseminated with X-sorted orunsorted sperm. Animal Reproduction 1:86-90.

Schenk JL, Cran DG, Everett RW, Seidel Jr GE (2009). Pregnancy rates in heifers and cows with cryopreserved sexed sperm: effects of sperm numbers per inseminate, sorting pressure and sperm storage before sorting. Theriogenology 71:717-728.

Soares JG, Martins CM, Carvalho NAT, Nicacio AC, Abreu-Silva AL, Campos Filho EP, Torres Junior JRS, Sa Filho MF, Baruselli PS (2011). Timing of insemination using sex-sorted sperm in embryo production with *Bos indicus* and *Bos taurus* superovulated donors. Animal Reproduction Science 127:148-153.

Tamrat D, Lemma A, Demissie E, Ali S, Funga A (2018). Superovulation Response and In vivo Embryo. Ethiopian Journal of Agricultural Sciences 28(1):71-80.

Tegegne A, Hoekstra D, Gebremedhin B, Gizaw S (2016). History and Experiences of Hormonal Oestrus Synchronization and Mass Insemination of Cattle for Improved Genetics in Ethiopia: From science to developmental impact. LIVES Working Paper 16. Nairobi,

Kenya: International Livestock Research Institute (ILRI).
Yehalaw B, Jemberu A, Asnake A, Wube A, Hirpa H (2018). Factors Affecting the Efficiency of Artificial Insemination in Dairy Cows in and Around Bishoftu (Debre Zeite), Oromia Regional State, Ethiopia. Journal of Reproduction and Infertility 9(2):28-35.