Influence of breed on the quality of *in vivo* produced embryos from Boran and Holstein Friesian cross dairy breed in Ethiopia

Hamid Jemal[1,2], Tamrat Degefa[3], Sayid Ali[3], and Alemayehu Lemma[1]*

[1]*College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O. Box 34, Bishoftu, Ethiopia*

[2]*Southern Agricultural Research Institute, P.O. Box 06, Hawassa, Ethiopia*

[3]*Debre Zeit Agricultural Research Centre, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia*

*****Corresponding author: jemalhamide@gmail.com*

Abstract

The variation of the dairy breed can determine the success of bovine embryo transfer by influencing the quantity and quality of *in vivo* embryo production. In this experiment, output and quality of *in vivo* produced embryos using semen of progeny tested Holstein Friesian (HF) sire in Boran and HF*Boran F_1 cross cows, and semen from purebred Boran sire in HF*Boran F_1 cross and Boran cows were evaluated. Boran (n=18) and HF*Boran cross (n=18) breed donor dams were superovulated using a previously optimized follicular stimulating hormone (FSH) (Pluset®) dose regimen: 650 IU for HF*Boran cross and 250 IU for Boran breeds. Each cow was flushed on Day-7 post insemination and embryos were evaluated for their developmental stages and quality. Superovulatory response rates were 88.9% and 83.3%, respectively, for Boran and HF*Boran with no significant (*P*>0.05) breed differences. Total recovery rates were relatively lower (56.5%) in Boran compared to in HF*Boran (67.4%). The mean (±SE) embryo flush outputs were 6.5±0.8 for Boran and 6.9±0.7 for HF*Boran with no significant breed difference. Recovery of a transferrable embryo was significantly higher (68.0%; *P*<0.05) in HF*Boran dam inseminated with HF sire semen. Boran cows yielded a significantly higher (*P*<0.05) proportion of unfertilized ovum (57.6 %) irrespective of the sire breeds. Comparatively, a higher number of degenerated embryos were produced by HF*Boran cows. This study demonstrated that the presence of breed-related differences in both the quality and quantity of *in vivo* produced Bovine embryos.

Keywords: Bovine; dam; embryo collection; embryo transfer; sire; superovulation.

Introduction

In vivo embryo production is a reproductive technology generally used to improve the number of offspring from potentially elite cows using multiple ovulatory embryo transfer techniques (MOET) (Phillips and Jahnke, 2016). This technology has got more acceptances worldwide as the best and ethically acceptable method for getting quality embryos (Bó and Mapletoft, 2014; Bó *et al*., 2019). Quality embryos are used for transferring to the recipients and it is used mainly for genetic improvement to compliment increasing the numbers of a particular breed or phenotype (Junqueira *et al*., 2018). This can be done by applying the MOET technologies which require the meticulous selection of valuable donors in perfect gynaecological conditions, application of appropriate superovulation technique, the use of good quality semen, and reproductively sound recipients, all of which have a direct influence on the outcome of the embryo transfer program (Phillips and Jahnke, 2016; Bó *et al*., 2019).

Different studies (Peixoto *et al*., 2007; Perez *et al*., 2016; Perez *et al*., 2019; Vizoná *et al*., 2020) have been carried out elsewhere on *Bos indicus* and *Bos taurus* breeds showed that there are variations in response to the superovulation. This is due to individual variations where some females consistently produce large numbers of embryos in response to superovulation than other females of similar age and management but different breeds may also perform variably (Guerreiro *et al*., 2014; Batista *et al*., 2016). There are shreds of evidence for the variations originated from the genetic and physiological differences of the donors. As a physiological parameter of variations, differences in follicular dynamics of Ethiopian Boran breed and their HF crosses were characterized previously in Ethiopia (Degefa *et al*., 2016 and 2018). Concerning the sire effect, in a more recent study, Lemma and Shemsu (2015) reported that semen quality is highly influenced by age and breed of AI bulls which could influence *in vivo* fertilization. Besides the genetic factor, it has been indicated that production status, feeding, and insemination techniques are among other factors that can affect the quantity and quality of embryos both in single and multiple ovulations (Jemal and Lemma, 2015; Mikkola *et al*., 2019). Typically Sartori *et al*. (2010) have found that milk production, body condition score, the type and quantity of feed intake, and heat stress are the main potential risk factors that affect the quality of embryos. Still further researches are ongoing on how to improve the production of good quality embryos in cattle at a molecular level (Safari *et al*., 2018; Manzi *et al*., 2019; Vizoná *et al*., 2020).

In general embryo transfer program in Ethiopia has been initiated by the international livestock research institute (ILRI) in 1991 but was discontinued until subsequent works by Degefa *et al.* (2016) and Tadesse *et al.* (2016). The MOET techniques applied today in Ethiopia are those developed for *Bos taurus* with variable results calling the need to develop breed-specific modifications for better results. Currently, Boran and HF*Boran crosses are widely used in Ethiopia and East African countries' dairy sectors. Subsequently, evaluation of the efficiency and effectiveness of embryo transfer (ET) in these breeds is very economical.

Ovarian dynamics and superovulatory response are partially governed by breed-related physiological differences. In this study, we hypothesized that subsequent embryo quality and recovery rate could also be influenced by breed differences either of the dam or the sire considering indigenous Boran breeds and their HF F_1 crosses.

Materials and methods

Study area and study animals

The study was conducted at Debre Zeit Agricultural Research Centre (DZARC) and College of Veterinary Medicine and Agriculture (CVMA). The study site is located at 08° 44' N and 38° 58' E (Latitude/ Longitude) at 1900 meters above sea level (m.a.s.l) with the average annual temperature ranging from 9.8°C to 28.3°C. It receives an average annual rainfall of 851 mm in a bimodal season (EIAR, 2020). A two-stage screening was carried out to assign eligible donors. From 135 (pure and cross) HF and 60 Boran herd, a pool of 28 cross HF and 21 pure Boran donor cows were targeted based on the history (record book) for their blood level, reproductive stage (non-lactating and at least one parity) and body condition score (BCS >3.5 on 1 - 5 scale) at the time of screening (Klopčič *et al.*, 2011). Selected animals were further subjected to gynaecological examination using ultrasonography and only those with intact CL (cycling cows) and without reproductive health problems (uterine fluid and sign of endometritis) were submitted for the experiment. All animals were managed under the same condition, provided with good housing, and fed on forage and concentrate supplements. Water was provided *ad libitum*. The donors received vaccination against common infections and were regularly dewormed as they were also allowed to graze.

Study design

The two-level Dam-Sire combination method for embryo production was designed and experimental animals were randomly assigned for Pluset® treatment. Imported high-quality purebred Boran semen and progeny tested HF-Friesian (HF) Sire semen was used. Semen from either of the bulls were randomly assigned and inseminated to donor cows as per the design. The overall procedure was conducted according to the four combinations of treatments using a 2x2 design as described below in Table 1.

Table 1. An arrangement of 2x2 dam sire combination model

Combination	Boran donor dam	HF*Boran donor dam
Boran Sire	TR1= 9	TR3= 9
HF Sire	TR2= 9	TR4= 9
Total	18	18

TR-Treatment

Treatment protocol

A total of 36 (Boran breed n=18) and (HF*Boran cross n=18) were superovulated. The HF*Boran cows were treated with an IM injection of FSH (Pluset®, Barcelona, Spain) at a dose rate of 650 IU (5.75ml) while the pure Borans were given 250 IU (2.5ml) of the same Pluset® as previously described by Degefa *et al.* (2018). The treatment schedule was a twice-daily injection of dispensed Pluset® over four days starting Day 4 after the insertion of CIDR (Progesterone 1.38 gm, Hamilton, New Zealand) in a decreasing volume as 2.5ml, 1.5ml, 1.25ml, 0.5ml, for HF*Boran crosses and, 1ml, 0.75ml, 0.5ml, 0.25ml for Boran. On Day six, each cow received an IM injection of 2 ml prostaglandin $F_2α$ ($PGF_2α$) (Cloprostenol Sodium, Germany) twice a day (b.i.d) while the CIDR was removed on Day 7. Animals were inseminated twice to attain the optimum concentration of semen for multiple ovulations upon the detection of standing estrus following the superovulation treatment. Besides, all flushed cows were injected 2 ml $PGF_2α$ to avoid further conception.

Recovery of embryos and evaluation

Embryos were flushed using a two-way Foley catheter with 1 litre of lactated ringer solution mixed with 1-5% calf serum on the 7^{th}-day post-AI and considering 500ml for each uterine horn. Donors received 3-5ml of epidural anaesthesia (2% Lidocaine) during the procedure. Superovulatory response was evaluated based on an ultrasonic count of the corpus luteum (CL). The embryos were evaluated for their developmental stage (from stage 1 = 1-cell to stage 9 = expanded hatched blastocyst) and for their quality (from Grade 1 = excellent to Grade 4 = degenerate/ dead) according to the International Embryo Transfer Society guidelines described in Jahnke *et al.* (2015) and Baruselli *et al.* (2006). Embryos were categorized as transferable (Grades 1, 2, and 3), freezable (Grade 1&2 only), Degenerate (Grade 4), Poor quality embryos (Grade 3 and 4), Unfertilized ovum (UFO).

The quality of the embryo was determined based on the following 4 parameters:

- Excellent- An ideal embryo, spherical, symmetrical, and uniform sized blastomeres, with uniform color
- Good- Few extruded blastomeres, slightly irregular shape, few vesicles
- Poor- Definite but no severe problem, extruded blastomeres, vesiculation of few degenerated cells
- Degenerated- Severe problems, numerous extruded cells, degenerated cells of different sizes, large vesicles with dark color

Response rate (%) was computed as the number of cows with at least three CL (Vieira *et al.*, 2014) and/or persistent follicles (PUF)/the Total number of treated cows*100.

Recovery rate (%) = Total recovery or flush output (UFO + embryos)/Total number of (CL+PUF) *100;

The proportion of UFO (%) = Number of UFO/Total recovery or flush output * 100;

Rate of transferable embryos = Total number of transferable embryos/ Total recovery or flush output* 100.

Endpoint definitions

- *Total recovery*- The recovery of the total substances (UFO and embryos) compared with the total ovulations in terms of the total CL and PUF counted
- *Total flush output*- The total average of recovered substances (UFO and embryos) per individual donor cow
- *Superovulatory response*- The extent of ovulation of preovulatory follicles in terms of CL and also the PUF
- *Persistent unovulatory follicles*- Those preovulatory follicles failed to ovulate and remaining persistent until days of embryo flush

- *The proportion of UFO*- The ratio of recovery of unfertilized ovum versus the *total flush output*

Statistical analysis

All data collected were summarized into a Microsoft Excel sheet. Skewed dependent variables of the superovulatory response (UFO, fertilized, transferable, freezable, degenerated) were transformed using the Zero-skewness log transform technique and tested for their significance effect and interaction terms using STATA® version 14.0. Poisson regression was run to test differences between variables (a breed of dam and sire), and embryo quality parameters. The effect of breed of the dam or sire was computed by Two-way factorial ANOVA and Bonferroni pairwise mean comparison test. The quality of embryos was evaluated by General Linear Model (GLM). The level of significance was held at $P<0.05$.

Results

Embryo production

Overall, 88.9% (n=16) of Boran and 83.3% (n=15) of HF*Boran donors responded to the superovulatory treatment. A total of 210 (3-18/ cow) and 191 (2-15/ cow) CL were observed in Boran and HF*Boran donors. The overall recovery rate was 56.7% and 67.4% in Boran and HF*Boran, respectively. 57.6% UFO collected over the total flush output (n= 117) from Boran donor dams while 17% (n=125) was from HF*Boran. Descriptive statistics of the superovulatory responses and embryo recovery outputs were summarized in both breeds (n=36) Table 2.

Table 2. Mean (± SE) of the superovulatory response and outputs of embryo flush in Boran and HF*Boran donor cows

Parameters	Boran (n=18)	HF*Bora (n=18)
CL count	11.6±1.0 (Range= 3 - 18)	10.6±0.9 (Range= 2 - 15)
Post unovulatory follicles	1.8 ± 0.4	1.4 ± 0.3
Total flush outputs (UFO + Embryo)	6.5 ± 0.8 (Range=1- 10)	6.9 ± 0.7 (Range=2- 10)
Unfertilized ovum (UFO)	3.6 ± 0.6	1.3 ± 0.3
Total embryos	2.8 ± 0.6	5.6 ± 0.6
Transferable embryos	2.3 ± 0.6	4.7 ± 0.6

Comparison of the Dam and/ or Sire effects

Comparison of donor and/ or sire effects on quality of recovered embryos shows significant effects of donors and to lesser extent sires and statistically no significant interaction effects. Individual effects were evaluated for the validation of their significance and have shown that dam significantly affect the quality of all recovery types than sire as described in Table 3.

Table 3. Two-way ANOVA of dam and sire effect on embryo quality

Embryo quality	Dam*			Sire			Dam^sire interaction		
	MS	F	*P* value	MS	F	*P* value	MS	F	*P* value
UFO	2.98	11.15	0.002	0.64	2.42	0.129	1.00	3.75	0.061
Fertilized	0.55	13.20	0.001	0.25	6.12	0.018	0.02	0.65	0.413
Transferable	0.82	9.98	0.003	0.37	4.48	0.042	0.08	1.06	0.310
Freezable	0.97	4.42	0.043	0.81	3.70	0.063	0.17	0.81	0.375
Degenerated	0.44	19.86	0.000	0.00	0.24	0.625	0.01	0.69	0.425

^ refers interactions; df=1; MS= Mean square; * significant association of dam to all outcomes than sire

Status of embryo qualities

All embryos were sorted into grades while freezable (Grades 1 & 2, and Stages 4-8) and poor-quality embryos (Grades 3 & 4) were computed for the dam-sire combination effects. Unlike the lower fertilized embryo counts in Boran cows, there was a higher count of G1 and G2 embryos from the overall recovered embryos regardless of the sire effect. Similarly, HF*Boran cows produce higher count G2 embryos than other quality grades in the breed contest (Figure 1).

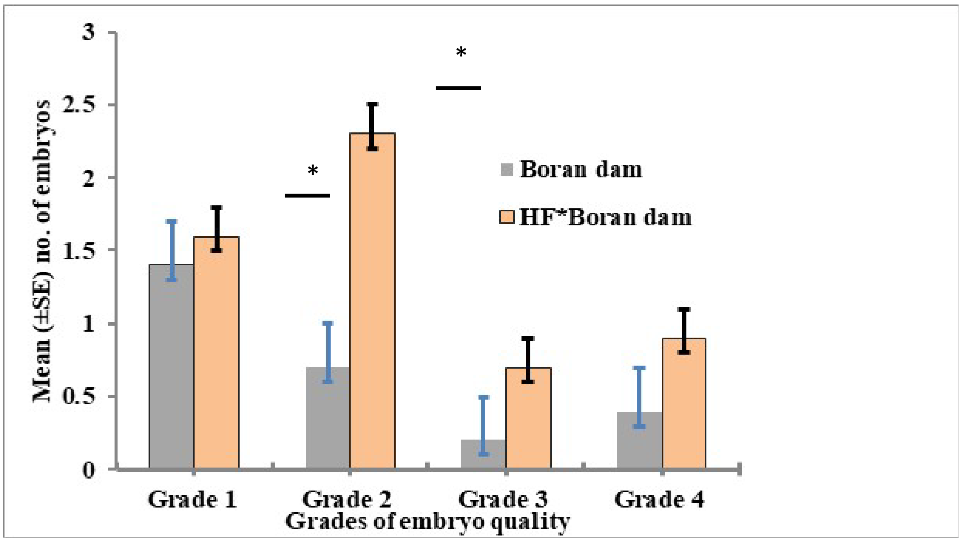

Figure 1. The overall quality of recovered embryos in both bred

On the other hand, when comparing each grade of embryo quality, HF*Boran dams inseminated with HF sire yield a higher number of excellent and good embryos (Grade 1 & 2) and also had yield embryos of poor-quality grades (Grade 3 & 4) than Boran cows inseminated with Boran sire (Figure 2).

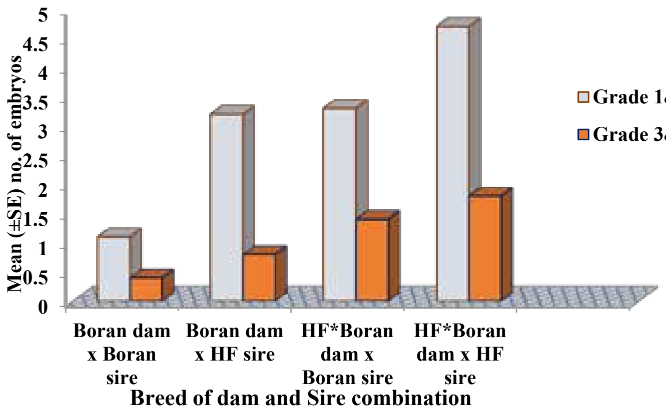

Figure 2. Classification of embryo qualities based on their dam-sire breed

Further analysis of different qualities of embryos recovered during alternate use of dam and sire breed showed a higher number of fertilized and transferable embryos when HF*Boran dams inseminated with either of the sires. Whereas a larger number of degenerated and poor quality embryos were found when HF*Boran donor dams were inseminated with HF sires, while a higher number of UFOs were recovered when Boran dams were inseminated with Boran sire (Table 4).

Table 4. The average number of different categories of embryo qualities resulting from the four dam-sire combinations of Boran and HF*Boran crossbreeds

Embryo category	Mean (±SE) number of embryos for dam sire combinations (x)			
	Boran dam x Boran sire	Boran dam x HF sire	HF*Boran dam x Boran sire	HF*Boran dam x HF sire
Fertilized	1.8 ± 0.8	3.8 ± 0.8	4.8 ± 0.8[a]	6.4 ± 0.8[b]
Transferable	1.2 ± 0.8	3.2 ± 0.8	4.1 ± 0.8	5.3 ± 0.8[a]
Freezable	1.2 ± 0.8	3.1 ± 0.8	3.3 ± 0.8	4.7 ± 0.8[a]
Degenerated	0.6 ± 0.8	0.7 ± 0.8	1.9 ± 0.8[a]	1.8 ± 0.8[a]
Poor quality	0.4 ± 0.3	0.8 ± 0.3	1.4 ± 0.3	1.8 ± 0.3[a]
UFO	3.9 ± 0.7[a]	3.3 ± 0.7	2.1 ± 0.7	0.6 ± 0.7

Superscripts significantly different at P<0.05 and 0.001, for the values "a" and "b", respectively

Discussion

In this study, the superovulatory response was not significantly different between donor breeds which agrees to previous reports by Batista *et al.* (2016), Degefa *et al.* (2016), and Degefa *et al.* (2018). However, other authors (Purohit *et al.*, 2013; Guerreiro *et al.*, 2014) showed that *Bos indicus* have an inherently low ovarian follicular population and respond less than the *Taurus* breeds. On the other hand, *Bos indicus* animals are said to be more sensitive to exogenous gonadotropins and respond better to superovulation (Ferreira *et al.*, 2014). This variation might indicate the presence of a more important individual variation rather than breed influence when it comes to superovulatory treatment in bovine (Curtis, 2015). The average CL detection was comparable to previous studies for the HF crossbreeds (Ferreira *et al.*, 2014; Vieira *et al.*, 2014; Tadesse *et al.*, 2016) though fewer numbers have also been reported (Hussein *et al.*, 2014). Several other studies (Peixoto *et al.*, 2007; Vieira *et al.*, 2014; Mapletoft *et al.*, 2015; Perez *et al.*, 2016; Perez *et al.*, 2019; Vizoná *et* *al*., 2020), confirmed that the variations in response to superovulation and recovery rate are not only due to breed differences but also the status of donors at the time of superovulation. In line with a report by Degefa *et al.* (2018) on similar animals, the Boran breed showed a comparable ovarian response as the HF*Boran, however, recovery of UFO was much higher in Boran cows compared to the HF*Boran crosses. A likely cause is fertilization failures were reported by several authors (Sartori *et al*., 2010; Rasolomboahanginjatovo *et al*., 2014; Dorice *et al*., 2019) where a higher proportion of non-fertilized oocytes and degenerated embryos have been frequently recovered. Fertilization failure in superovulated cattle is generally more pronounced, averaging approximately 45%, much lower than the present finding. It is mainly associated with poor gamete transport due to hormonal imbalances or suboptimal oocyte quality which is not uncommon in zebu breeds (Dorice *et al*., 2019). Hasler (2012) in his study in Red Angus beef breeds reported a closely similar proportion of UFO and a higher proportion of degenerated embryos in superovulated cows.

The mean number of transferable embryos found in the present study is much higher compared to a previous report by Tadesse *et al.* (2016) from a similar HF*Boran crossbreeds. However, it was smaller than those reported for Nelore breeds of donor cows where local variability was confirmed to be due to farm management and donor age as the main factors (Silva *et al*., 2009). A closer analysis of the effect of the dam-sire combinations in this study confirms the insemination of Boran dam with Boran sire to be the source of the largest proportion of UFOs recovered. This finding is in agreement with Degefa *et al.* (2018) where a higher yield of UFO was recorded in Boran cows inseminated with semen from Boran sire. Probable causes for the high proportion of UFO in Boran cows might be related to the breed itself or the condition of the dam including nutritional status at the time of insemination (Ferreira *et al*., 2014). Sire effects are also related to fertilization ability, semen quality and dose, immunogenetics, and other related factors which might contribute to the variation (Hasler, 2012). However, the use of high-grade imported Boran semen in our study has not significantly altered the incidence of UFOs from Degefa *et al.* (2018) who used both AI and the natural service of indigenous Boran bulls. It is imperative to assume then the likely source of higher incidence of UFO is probably the oocyte quality than the semen. On the other hand, a significantly larger proportion of degenerated embryos were harvested from HF*Boran donor cows in the current study. Hasler (2012) and Trigal *et al.* (2012) reported that the male effect is more evident on the variation of superovulatory outcomes in beef breeds and from using sex-sorted semen. Contrary to this, the crossing of HF*Boran dam with semen from pure HF bulls produced a significantly higher proportion of transferable embryos relative to the Boran dams in all possible combinations. This probably shows the role of the donor dam breed which is also confirmed previously by Degefa *et al.* (2018) though the overall result was comparatively smaller in our study. More yield of freezable embryos was collected than is reported by Vieira *et al.* (2014) while the average number of fertilized embryos from HF*Boran cows is comparable. The HF*Boran donor cows produced a significantly higher number of Grade 1-3 quality embryos compared with the Boran breed in this study. Different kinds of literature (Alarcón *et al.*, 2010; Trigal *et al.*, 2012; Vieira *et al.*, 2014; Tadesse *et al.*, 2016; Chinchilla-Vargas *et al.*, 2018) suggest the source of these variations to be related to breed, reproductive behaviour, environmental factors or interaction of all.

Conclusions

It can be concluded that the recovery rate of the embryo might be comparable for the two breeds in any combination of the dam and sire we compared. However, a higher incidence of UFO was evident in Boran cows irrespective of the sire breeds while a higher number of degenerated embryos were produced by HF*Boran cows. Further, both the quality of the embryo and the proportion of transferable embryos are associated to breed differences. Interestingly, comparatively more transferable embryos were recovered when semen from pure HF sire was used in both breeds of the dams.

Conflict of interest

The authors declare that they have no conflict of interest

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