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Superovulation Response and *In vivo* Embryo Production Potential of Boran Cows in Ethiopia

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Abstract

*Boran (n=25) and Boran*Holstein (n=11) cows were superovullatedFSH with three doses level (300, 250 and 200IU) divided in to morning and afternoon decreasing doses over 4 daysto study the superovulatory response and embryo production potential. Time to estrus, duration of estrus, and CL count were used to determine superovulatory response. Embryos were flushed on Day 16/17 by non-surgical gravitational method and evaluated for development stage, and quality grade. The mean (±SEM) time interval from CIDR withdrawal to onset of estrus was 20.4±1.8 hours, and breed difference was not significant.However, the interval from CIDR removal to onset of estrus was shorter (p=0.01) in cows treated with 250 IU FSH (10.75±3.3 hours) than in cows received 200 or 300 IU. The total CL count was significantly higher (p=0.01) in Boran (10.1CL/cow/cycle) than Boran x Holstein cows (7.2CL/cow/cycle). A mean number of 4.1 and 2.67embryos'/cow were flushed from Boran and Boran*Holstein, respectively. The average transferable embryos/cow were 3.8±0.57 and 2.67±0.99 in Boran and Boran*Holstein, respectively. And hence, Boran cows' response to superovulation and yield of better quality and number of embryo than their Boran*Holstein counterparts showed the high potential of the breed for in-vivo and in-vitro embyo production.*

Introduction

Boran cattle are the most suitable types of breed for arid and semi-arid regions due to their adaptive characteristics: tolerance to heat, ticks' infestation, feed and water shortage (Solomon, 2001). Different literatures showed that they are superior to other highland cattle in Ethiopia in birth weight, weaning weight, faster growth rate, mature weight for cows, higher fertility, daily milk yield, milk yield/lactation, lactation length, milk fat, age at first calving, calving interval, and calving rate, (Mekonnen *et al.,* 2010). They have large body size with good body conformation (large and long-legged, tall height, broad back and wide pin-bones, well-developed dewlap and udder, and well-developed hindquarters) and body weight ranging from 318 - 680 kg in male and 225 - 454 kg in female (Mekonnen *et al,* 2010). Except one trial Boran breed showed better potential for superovulation and embryo production (yielded more numbers of embryos/ova and transferable embryos than BoranxHolstein cows; Tegegne *et al.,* 1997) the effort made so far to increase the productivity on Boran cattle in Ethiopia was fully based on crossing with exotic breeds either through AI or natural breeding.

On the other hands, some reports on reproductive physiology of Zebu cattle indicated that their sensitivity to gonadotropins, estrus behavior and ovulation moment, timing of estrous expression, number of follicular waves during estrous cycle,number of total follicles,size of follicular diameter at deviation and luteal tissue size are not similar to that of *Bostaurus* breed cows (Baruselli*et al.,* 2006). These variables are largely studied in temperate breeds, but very limited on Zebu cattle breeds (Figueiredo *et al.,* 1997). Knowledge of these parameters undoubtly enhancesthe application of reproductive technologies for conservation and sustainable utilization of these genotypes. Therefore,the study was made to assess the superovulatory response and quality of *in vivo* produced embryos of purebred Ethiopian Boran in comparison to their Holstein crosses.

Materials and Methods

The experiment was carried out at DebreZeit Agricultural Research Center, EIAR,located about 45 km east of Addis Ababa, the capital city of Ethiopia (8°46′13.57"N, 38°59′50.45"E) at an altitude of 1920 masl.

Experimental animals

Boran and Boran*Holstein cows in their first and second parity were used for this study. Breeding and clinical records of all cows were reviewed, underwent a thorough reproductive examination, and only cows free of reproductive abnormalities were included. Selected cows had a mean body condition score (BCS) of 7 (on a scale of 1 to 9; 1=emaciated; 9 =obese). All cows were managed under uniform management and housing system, provided with feed of different mix: *tef* straw (*Eragrostistef*) and grass hay (*Andropogonabyssinicus*) as a basal diet and supplemented with commercially prepared concentrate, mineral salts and alfalfa green fodder up to a limit of 3% life body weight per cow.

A total of 36cows (25 Boran and 11 Boran x Holstein crossbreds) between 6 and 8 years of age(previously serially scanned for 61 days using ultrasoundfor ovarian dynamics) were selected for the superovulation treatment regime illustrated in Figure1. CIDR were implanted in both genotypes (Progesterone 1.38 gm, Hamilton, New Zealand) for seven days (Day 0 = date of CIDR insertion). On Day 4 cows were randomly allocated into one of the three FSH treatment groups: 300 IU, 250 IU, or 200 IU (Pluset®, Spain). FSH was administered in a series of eight intramuscular injections given over four days in a decreasing dose regimen (Table 1) and injected with PGF2α analogue (Estrumate®, France) IM on Day 6, and the CIDR was withdrawn on Day 7 at 6 PM concurrent with the last injection of FSH.

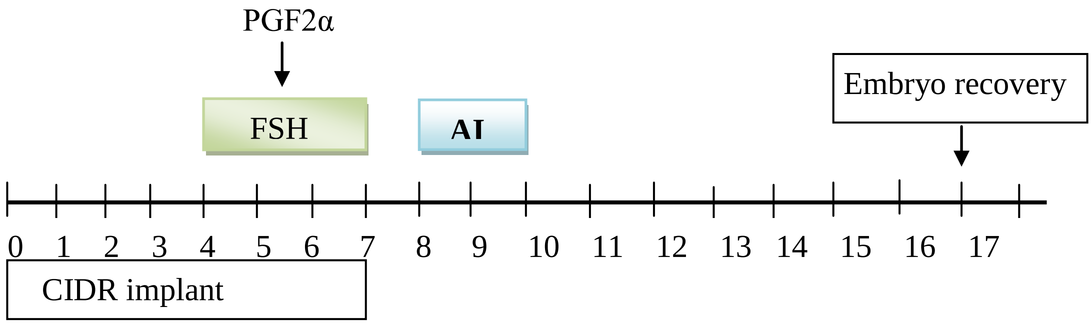

Figure 1. Superovulation and embryo recovery protocol used in Boran and Boran*Holstein cow

Table 1. Three FSH dose (IU) treatment groups for superovulation of Boran and Boran*Holstein cows

Group	No	Dose (IU)							
		Day 4		Day 5		Day 6		Day 7	
		Am	Pm	Am	Pm	Am	Pm	Am	Pm
300	14	60	60	45	45	30	30	15	15
250	8	50	50	35	35	25	25	25	25
200	14	40	40	30	30	20	20	10	10

All cows were meticulously monitored to determine the onset and duration of estrus until the last cow exhibited estrus. Cows were inseminated twice (morning and afternoon) based on the manifestation of standing heat to capture the possibility of extended ovulation. In addition to recorded time from CIDR removal to onset of estrus and duration of estrus, cows were subjected to ultrasonic (CTS3300, Altay Scientific, Italy) evaluation to determine superovulatory response based on total number of CL and unovulated follicles present on both ovaries on Day 17before embryo recovery. Non-surgical closed gravity flow embryo recovery method was performed with commercial flushing medium (ViGRO™, Bioniche Animal Health, USA) and using a two-way Foley catheter (18 Fr 650 mm length; MOFA®, Canada) to collect the embryos.

Embryos evaluation and grading

Harvested embryos and/or unfertilized ova were transferred from embryo filter to a search dish containing holding medium and grouped in to embryos and UFO under a stereomicroscope (MotisSMZ 140/143®, Roanoke, USA). The embryos were graded for their developmental stage (from stage 1 = 1-cell to stage 9 = expanded hatched blastocyst) and quality (from quality 1 = excellent to quality 4 = degenerate) according to the standards given by the International Embryo Transfer Society (IETS). IETSgrading categorize developmental stages 4-8 with a quality grade of 1 or 2 were considered transferable, andconsiderall others as non-viable.

Descriptive statistics and univariate analysis of the SAS were used to analyze the genotype response to FSH treatments (in terms of unovulated follicles and CL). Frequency distribution, chi-square test and one-way ANOVA were used to analyze the data for rate of embryo recovery and results of laboratory evaluation.The level of significance was held at P<0.05.

Results and Discussion

The total number of ovarian follicles is indicated in Table 2, and no breed difference was observed.

Table 2. Total number of follicles ≥ 4 mm diameter in Boran and Boran*Holstein cows

Breed	N	Total follicles ≥ 4 mm on Right ovary Mean (±SEM)	Total follicles ≥ 4 mm on Left ovary Mean (±SEM)	Total follicles ≥ 4 mm on Both ovaries Mean (±SEM)
Boran	9	9.14±0.091 (Range=4-17)	9.20±0.083 (Range=5-14)	18.34±0.145 (Range=11-28)
Boran*Holstein	8	8.69±0.089 (Range=5-17)	8.87±0.084 (Range=4-14)	17.56±0.141 (Range=10-28)
Total	17	8.93±0.064 (Range=4-17)	9.05±0.059 (Range=4-14)	17.98±0.102 (Range=10-28)

Superovulatory response

The overall response to superovulatory treatment was 76.6%, but response was higher (92.6%)in Boran (χ^2 = 9.057; p=0.003) than the 55.0% in Boran* Holstein cows. The mean (±SEM) time interval from withdrawal of the CIDR to onset of estrus was 20.4±1.8 hours, and was not affected by genotype (18.6±2.10 hours in Boran and 19.75±2.88 hours in BoranxHolstein; Table 3). However, the interval from CIDR removal to onset of estrus was shorter (p=0.01) in cows treated with 250 IU FSH (10.75±3.3 hours) than in cows receiving either 200 or 300 IU. The mean duration of estrus was 22.9±1.1 hours, and duration of estrus was shorter (p=0.005) for cows treated with 250 IU FSH than for cows receiving 200IU or 300IU.

Table 3. Time interval from CIDR withdrawal to onset of estrus [hrs.] and duration of estrus [hrs.] in Boran and Boran*Holstein cows treated at different doses of Pluset

Breed of cows and dose of FSH	N	FSH dose [IU]	Mean (±SEM) Interval (hr)	P - value	Mean(±SEM) Duration of estrus (hr)	P -value
Boran	25		18.6±2.1	NS	21.8±1.4	NS
stein	11		19.8±2.9		22.8±1.9	
FSH	14	200*	22.6±3.1		26.1±1.9[a]	
	8	250**	10.8±3.3		16.8±2.1	
	14	300**	24.2±2.8		24.0±1.8[b]	
Boran	11	200	23.2±2.9	NS	24.9±1.7	NS
	4	250	10.8±4.7		15.5±2.9	
	10	300	21.8±2.9		24.4±1.8	
Boran x Holstein	3	200	22.0±5.5		27.3±3.4	
	4	250	10.8±4.7		17.0±2.9	
	4	300	26.5±4.7		24.0±2.9	

** The mean difference was significant at p=0.007; ** the mean difference was significant at p=0.006;*
[a] mean difference was significant at p=0.003; [b] the mean difference was significant at p=0.01

The mean (±SEM) number of CL counted ultrasonographically was 8.6 ± 0.7, and the maximum number of CL counted (17 CL) was detected in a Boran cow treated with 250 IU. Table 4 shows the number of CL by genotype and by pluset dose.

Table 4. The number of CL counted by ultrasound for purebred Boran and Boran x Holstein crossbred cows receiving different doses of exogenous FSH

Breed	N	No of CL by Ultrasound	
		Mean±SEM	P-value
Boran	25	10.1±0.7	0.01
lstein cross	11	7.2±0.9	
FSH level (IU) 200	14	7.0±0.9	0.05
250	8	10.6±1.1	
300	14	8.3±0.9	NS
Boran 200	11	8.0±0.9	NS
250	4	13.8±1.5	NS
300	10	8.6±0.9	NS
Boran x Holstein crosses 200	3	6.0±1.7	NS
250	4	7.5±1.5	NS
300	4	8.0±1.5	NS

In vivo embryo production

Thirty-nine flushing'swere made on 10 Boran and 10 Boran*Holstein with a total yield of 118 embryos/ova's. Boran cows were flushed 1-4 time while Boran*Holstein were flushed 1-2 times. Sixty-five percent of (N = 25) of flushing was done on Boran cows yielding 76.8% transferable embryo (Plate 1and 2) and 49 UFO while it yielded 23.2% of the transferable embryo and no UFO from Boran*Holstein cows. The total collection of embryo/ova from Boran and

Boran*Holstein cows was 5.36±0.86 embryo/ova per cows (Table 5). Significant (p=0.001) genotype effect was seen on the total collection.There was a higher number of embryo/ova (P=0.001) recoveries from Boran than from Boran*Holstein cows. More UFO (Plate 3) were collected from Boran cows (mean ± SEM = 8.17 ± 1.82) than from Boran*Holstein cows (no UFO recovered).

All collected embryos were light amber colored cytoplasm, round shape, distinguishable blastomeres, wider per vitelline space, well defined inner cell mass (in blastocyst stage embryos), and clear and transparent zonapellucida which is an evident in the top quality embryos.The mean number of transferable quality embryos (TQE) produced was 3.45 ± 0.50, and this was not different between the genotypes (3.79 ± 0.57) for Boran, and 2.67 ± 0.99 for Boran*Holstein). Though it was not statistically significant, the mean number of unovulated follicles tended to be higher in Boran*Holstein cows (2.41 ± 0.59) than in purebred Boran cows (1.59 ± 0.28).

Among the TQE 71.4% were quality grade 1 whereas the remaining 28.6% were quality grade 2 (Plates 1, 2, and 4). Embryonic stages of development of harvested TQE were stage 4, 6 and 7 (Plates 1 and2). Six fresh embryos (4 stage 4 and quality grade 1, and 2 stage 7 and quality grade 1) were transferred to synchronized recipients of which fourpregnancy established and three live calves were born (Plate 5).

Table 5. Mean number of embryos/UFO and transferable embryos flushed from Boran and Boran*Holstein cross cows

Breed	Counts	N	Mean±SEM	Range
Boran	CL number	25	**9.80±0.57**	3 - 17
	Embryo yield	14	**3.79±0.57**	1 - 8
	UFO	6	**8.17±1.82**	3 - 14
	Total collection	16	**6.38±1.03** **	1 - 16
Boran*Holstein cross	CL number	14	**5.79±0.90**	1 - 11
	Embryo yield	6	**2.67±0.99**	1 - 7
	UFO		**0.0**	0
	Total collection	6	**2.67±0.99** *	1 - 7
Total	CL number	39	8.38±0.58	1 - 17
	Embryo yield	20	3.45±0.50	1 - 8
	UFO	6	8.17±1.82	3-14
	Total collection	22	5.36±0.86	1 - 16

** Significantly different by genotype (p=0.01)

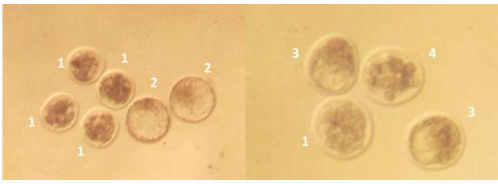

Plate 1. Development stage and quality of in vivo produced Boran: 1 = Stage 4 (Compacted morulla) quality1; 2 = Stage 7 (Expanded blastocyst) quality 1; 3 = Stage 6 (blastocyst) quality 1; 4 = stage 4 (compacted morulla) quality 2

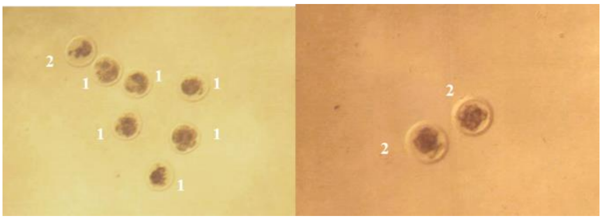

Plate 2. Development stage and quality of in vivo produced Boran*Holstein embryos: 1 = Stage 4 (Compacted morulla) quality 1; 2 = stage 4 (compacted morulla) quality 2

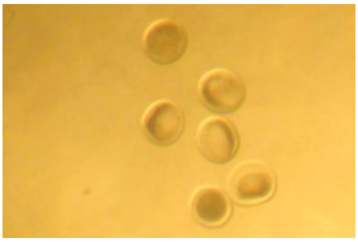

Plate 3. Unfertilized ovum collected from Boran

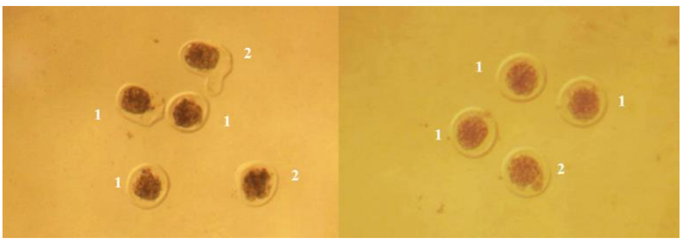

Plate 4. Embryos at the same development stage but different quality grade: 1 = Stage 4 (compact morula), Quality grade 1; 2 = Stage 4 (compact morula), Quality grade 2

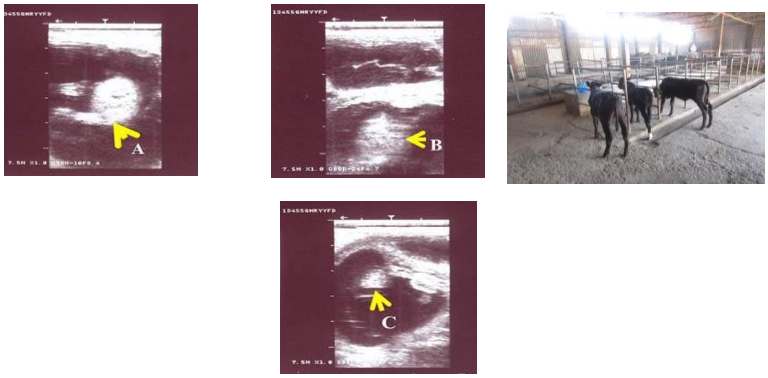

Plate 5. Sonogram of early pregnancy establishment from fresh embryo transfer (A, B, C) and calves born from the three pregnancies

Superovulation response of Boran cows

Genotype was found to be an important factor that influenced total number of ovarian follicles, follicular dynamics (Tamrat*et al*., 2016, Gimenes et al., 2009) and superovulatory response. Pure Boran was more sensitive to FSH dose and produced relativelylarge number of CL. Previous reports in other African cattle breeds such as the Cheurfa breed in Algeria (Ferrouk *et al.,* 2008)and N'dama breed in west Africa (Jordt *et al.,* 1986) confirm Boran to be superior. This could further be an indication of better performance of Boran in tropical environment than these African breeds, and greater sensitivity to exogenous gonadotropins, and is in complete agreement with Kanitz *et al., (*2002) who also studied dose and genotype interaction on the response to superovulation. The number of palpable CL recorded in the present study was substantially higher compared with a previous report on the same breeds treated with higher doses of "Pluset" (Tegegne *et al.,* 1997).

Previous reports had shown that an effort to increase the number of ovulations by increasing the FSH doses beyond the optimal level for a given breed did not increase superovulatory response except some undesirable results (Kanitz *et al.* 2002). Thesame phenomenon was observed in pure Boran treated with300 IU FSH. It wasconcluded that higher doses of FSH may disturb the process of ovulation by making ovaries too large or by inducing extremely high levels of estradiol synthesis (Kanitz *et al.,* 2002). Hence, this assumption holds true for Boran as well, and optimization of the superovulatory agent has a paramount importance for the wider application of the technology.

The quality of produced embryo (TQE) from Boran were consistent with previous findingfrom the same genotypes (Tegegne *et al.,* 1997), from the Algerian Cheurfa(Ferrouk *et al.* 2008), from the "N'dama" (Jordt and Lorenzini, 1990)in in terms of number of transferable quality. However, it was much lower than the result reported by Silva *et al.* (2009) for the Nellore (zebu) breed in Brazil. Although the difference was not significant the qualities of embryos collected from Boran were better than embryos from Boran*Holstein cows by all evaluation characteristics.All showed evidence of the top quality embryos: Light amber colored cytoplasm, round shape, distinguishable blastomeres, wider per vitelline space, well defined inner cell mass (in blastocyst stage embryos), and clear and transparent zonapellucida. This finding is in agreement with the defined character of excellent and good quality *in vivo* produced bovine embryos (Contreras *et al.*, 2008).

Several factors are known to cause collection of UFO from superovulated cows: cow factors such aslactation, seasonal effect, repeat-breeding, insemination at the onset of estrus, suboptimal oocyte quality (Contreras *et al.*, 2008), and increased doses of gonadotropin (Mapletoft *et al.*, 2002); inferior quality semen, disturbances in transport of spermatozoa and/or insemination with sex sorted spermatozoa (Kafi and McGowan, 1997). The most probable reason for the current situation was the inferior semen quality of the pure Boran bull procured from the National AI center.

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