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Ovarian follicular dynamics in purebred and crossbred Boran cows in Ethiopia

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Boran is an endangered breed of cattle indigenous to Ethiopia and the relatively poor understanding of its reproductive physiology has impeded efforts to maximize reproductive performance of the breed. This study characterized ovarian follicular dynamics in 9 purebred Boran and 8 Boran×Holstein (B×H) crossbred cows. Ovaries of all 17 cows were examined once per day for 61 consecutive days (encompassing three periods of estrus) using transrectal ultrasonography. The mean (±standard error of mean) inter-ovulatory interval (IOI) was similar (P>0.05) in Boran (19.4 ± 0.2 days) and B×H cows (20.1 ± 0.4 days). Two (in 79% of estrous cycles) or three (in 21% of cycles) follicular waves per IOI were observed and IOI was shorter (P<0.001) for cows with two follicular waves per estrous cycle (n=27; 19.5 ± 0.9 days) than for cows with three (n=7; 20.9 ± 2.1 days). All Boran cows (100%) exhibited two waves of follicular growth per estrous cycle and this was higher (P<0.001) than the proportion observed in B×H cows (56%). Mean diameter of the dominant follicle was similar (P>0.10) in both genotypes (15.8 ± 1.5 mm in Boran and 19.4 ± 2.9 mm in B×H). Boran cows possessed a greater (P<0.001) total number of ovarian follicles than B×H cows and both genotypes displayed more (P<0.05) activity on their right than left ovary. Results of our study have provided novel insights into the normal reproductive physiology of the Boran breed.

Key words: Boran, estrous cycle length, follicular dynamics, follicular waves, dominant follicle.

INTRODUCTION

Various researchers have documented that zebu cattle (*Bos indicus*) show several morphological and physiological differences from European taurine cattle. Follicular dynamics, one of the most important subjects in ovarian physiology, has been studied mostly in European breeds (Savio et al., 1988; Sirois and Fortune, 1988;

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Ginther et al., 1989; Roche and Boland, 1991; Badinga et al., 1994). The appropriate manifestation of estrus resulting from normal ovarian follicular dynamics is crucial to successful reproductive performance in cattle. A strong understanding of basic reproductive physiology enables appropriate application of controlled reproduction techniques (such as synchronization of estrus, synchronization of ovulation, and embryo transfer). Use of these reproductive biotechnologies can bring about sizeable improvements in the biological and economic efficiency of cattle breeding. Ultrasonography has enhanced the scientific understanding of the basic physiological processes in the estrous cycle including growth and regression of ovarian follicles and ovulation (Zacarias et al., 2015).

Studies in *Bos taurus* cattle have indicated the occurrence of two to four ovarian follicular waves during the estrous cycle; two waves are predominant, but four waves are rare (Sirois and Fortune, 1988; Rhodes et al., 1995; Bo et al., 1995; Adams, 1999; Townson et al., 2002; Evans, 2003; Sartori et al., 2004). Information available for zebu cattle showed a predominance of three ovarian follicular waves per estrous cycle with some observations of two, four, and even five (Zeitoun et al., 1996; Figeuiredo et al., 1997; Gambini et al., 1998; Viana et al., 2000; Mollo et al., 2007). Ethiopian zebu cattle exhibit a short duration/low intensity period of estrus, have a complete absence of behavioral estrus/receptivity to a bull in some cases, may refrain from repeated breeding attempts, and often ovulate without displaying overt signs of estrus (Tegegne et al., 1989; Tegegne et al., 1991; Bekele et al., 1991). Specific information on ovarian follicular dynamics in the Boran and other indigenous cattle breeds in Ethiopia, however, is absent. The objective of this study was to characterize ovarian follicular dynamics in purebred Boran and F_1 crossbred Boran cattle (created previously by insemination of Boran cows with Holstein semen in a national program aimed to increase milk production). It was hypothesized that purebred Boran cattle, adapted to Ethiopian conditions for centuries and reported by farmers as having greater fertility than crossbreds, would exhibit a different pattern of ovarian follicular waves than Boran×Holstein crossbred cows.

MATERIALS AND METHODS

This study was carried out for three consecutive cycles at the Debre Zeit Agricultural Research Center (DZARC) of the Ethiopian Institute of Agricultural Research, located about 45 km east of Addis Ababa (8°46′13.57"N, 38°59′50.45"E; 1900 m above sea level). The average annual temperature for the last five years was 18.5°C and average annual rainfall was 757 mm (DZARC Agro-meteorology, 2015). Seventeen cows (9 purebred Boran and 8 Boran×Holstein [B×H] crossbred) were used for this study after screening for normal reproductive anatomy through clinical and gynecological examinations. To monitor ovarian follicular dynamics, all cows underwent transrectal real-time B-mode ultrasonographic examinations using a 5.0 to 7.5 MHz linear array rectal transducer (Mindray, Hong Kong). The diameters of the three largest follicles were measured using the internal electronic calipers. Follicle sizes were categorized as small (1 to 3 mm), medium (4 to 6 mm), or large (≥7 mm) as previously described (Muasa, 2010). All follicles ≥4 mm diameter were counted on each ovary to determine follicular populations. The inter-ovulatory interval (IOI) was defined as the number of days between two consecutive ovulations in the same female. Ovulation was confirmed based on the sudden disappearance of the largest diameter follicle and subsequent appearance of a corpus luteum (CL) whose diameter was measured. The growth rate of the dominant follicle was computed from the date of divergence until ovulation.

Data were summarized using descriptive statistics and analysis of variance (PROC MIXED; SAS, 2004) was used to determine genotype effects. Mean comparisons were made using the least significant difference (LSD) procedure. Kendall's tau was computed to show rank correlations using the PROC CORR procedure of SAS. Differences were considered statistically significant at a P-value of $P<0.05$.

RESULTS

The mean (±standard error of mean [SEM]) IOI was 19.8 ± 0.2 days (n=34) and was not affected by genotype ($P>0.10$). Table 1 depicts results (segregated by genotype) of IOI and other parameters associated with ovarian follicular dynamics. Forty-five percent Boran cows had an IOI of 20 days (22% had 18 days, 22% had 19 days, and 11% had 21 days), whereas 38% of B×H crossbred cows had a 21-day IOI (range of 18 to 22 days).

The cows in this study exhibited two or three follicular waves during the IOI (Figures 1 and 2). A higher ($P=0.002$) proportion of Boran cows (100%) exhibited two follicular waves per estrous cycle than B×H crossbreds (56%). The remaining 44% of B×H crosses exhibited three follicular waves per estrous cycle; there were none with four or five. The mean (±SE) IOI for cows with two waves per cycle was 19.5 ± 0.9 days (Figure 3) and was shorter ($P=0.001$) than the 20.9 ± 2.1 days exhibited by cows with three waves.

The mean (± SEM) maximum diameter of the largest ovarian follicle was 17.0 ± 2.9 mm and this diameter was not affected ($P>0.10$) by genotype (16.9 ± 1.5 mm for Boran and 17.1 ± 2.9 for B×H crosses) (Table 2). However, purebred Boran cows had more ($P<0.001$) ovarian follicles than B×H crossbred (Table 2 and Figure 3), despite having a similar range in total number of follicles per cow (11 to 28 for Boran and 10 to 28 for B×H crosses). More ($P<0.001$) follicles were found on the right than the left ovaries in both breeds (Table 3). Representative ultrasonographic images of the ovaries of purebred Boran cows are shown in Plate 1.

DISCUSSION

Ultrasonography has been a highly useful tool to study reproductive events such as follicular wave emergence, dominant follicle selection, and ovulation (Gimenes et al.,

Table 1. Inter-ovulatory interval, number of follicular waves, and diameter of the dominant and subordinate follicles in purebred Boran and Boran×Holstein crossbred cows.

Parameter evaluated	Breed	N	Mean (±SEM)	Range
Inter-ovulatory Interval (IOI) [days]	Boran	18	19.44±0.23	18-21
	Boran×Holstein cross	16	20.13±0.35	18-21
Diameter of preovulatory dominant follicle (mm)	Boran	18	16.28±0.71	14-27
	Boran×Holstein cross	16	17.75±0.29	15-20
	Overall	34	16.97±0.415	14-27
Diameter of dominant follicle at day of divergence	Boran	18	9.28±0.311	8-13
	Boran×Holstein cross	16	9.75±0.348	8-12
	Total	34	9.50±0.232	8-13
Growth rate (mm/day)	Boran	18	1.14±0.15	0.50-3.2
	Boran×Holstein cross	16	1.46±0.12	0.71-2.3
	Overall	34	1.29±0.10	0.50-3.2
Diameter of largest subordinate follicle at ovulation (mm)	Boran	18	9.17±0.493	7-16
	Boran×Holstein cross	16	8.88±0.315	6-11
	Total	34	9.03±0.297	6-16
Diameter of second largest subordinate follicle at divergence (mm)	Boran	18	7.39±0.293	6-10
	Boran×Holstein cross	16	7.44±0.223	5-8
	Total	34	7.41±0.185	5-8

SEM, Standard error of mean.

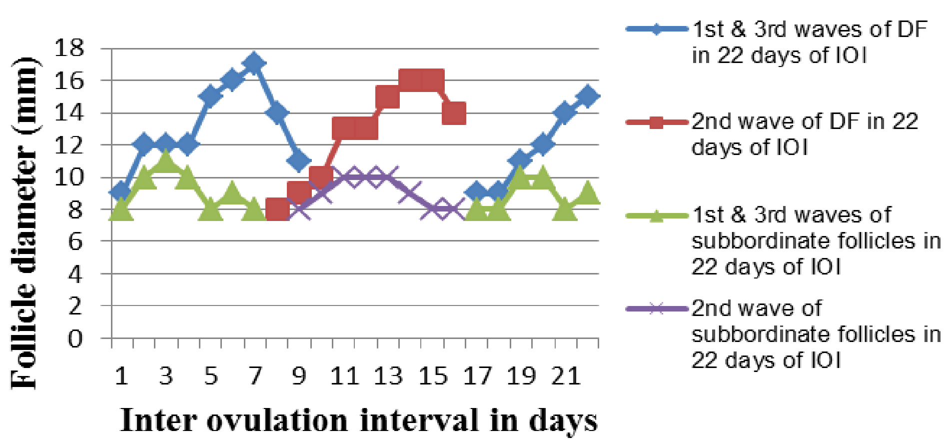

Figure 1. Ovarian follicular growth patterns of Boran×Holstein crossbred cows exhibiting three follicular waves per estrous cycle.

2008). A major benefit of ultrasound is that it facilitates the study of reproductive function without interrupting or distorting that function (as can occur when studying the same events via laparotomy or laparoscopy). The use of ultrasonography was reported herein to obtain the most detailed data recorded at present on ovarian follicular dynamics of the indigenous Ethiopian Boran breed of cattle.

The IOI for purebred Boran cows in the current study was shorter than the IOI reported by Bo et al. (2003) for in a review of *B. indicus* reproductive performance and by Sartori et al. (2004) for Nelore cows in Brazil. The IOI of

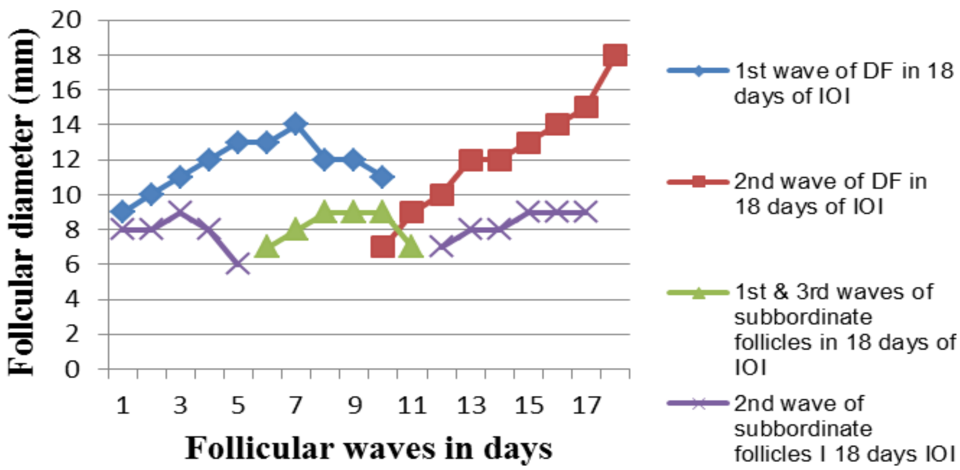

Figure 2. Ovarian follicular dynamics in purebred Boran & Boran*Holstein crossbred cows exhibiting two follicular waves per 18-day inter-ovulatory interval (IOI).

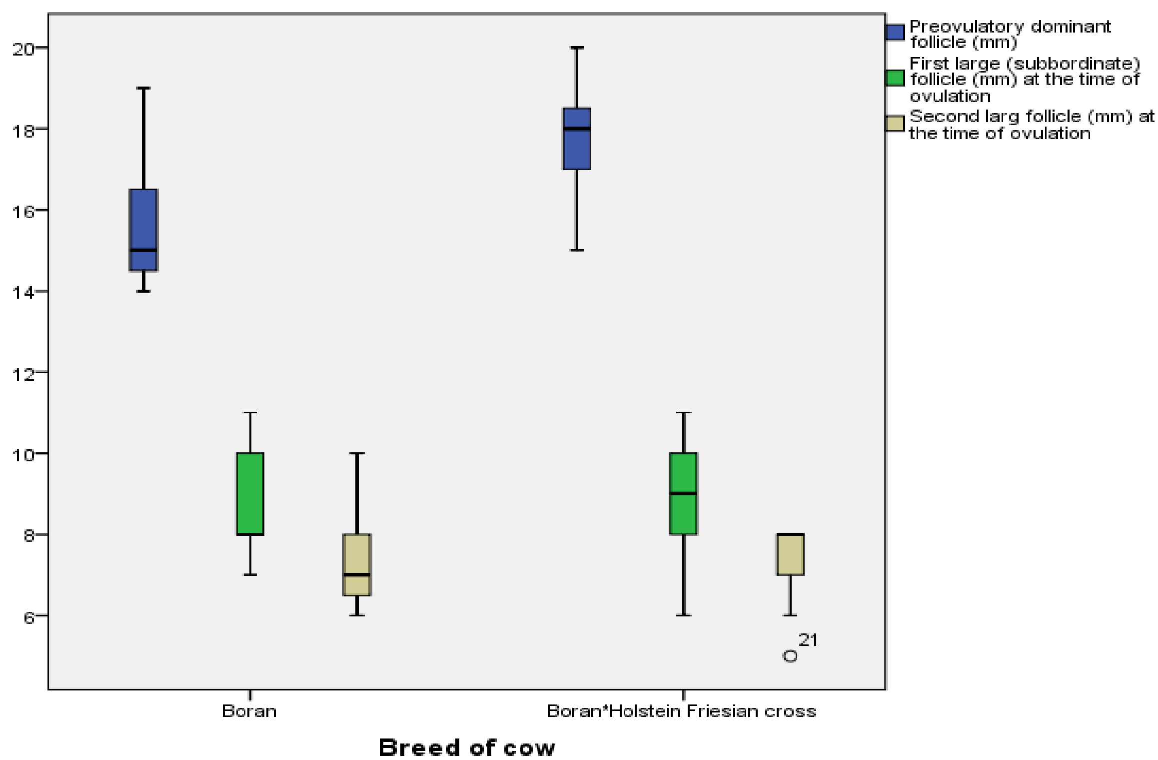

Figure 3. The size of the preovulatory dominant follicle (DF), largest subordinate follicle and second largest subordinate follicle in purebred Boran and Boran×Holstein crossbred cows.

the Ethiopian Boran in our study was also shorter than that reported for Kenyan Boran (Muraya, 2013), suggesting adaptation of the Boran to differing climates in these neighboring east African countries and/or a greater incidence of Kenyan Boran cows with three follicular waves per estrous cycle. The IOI of Nelore heifers in Brazil (Corte et al., 2012), however, was quite similar to our findings.

Early studies on ovarian follicular dynamics in *B. taurus* cattle (Savio et al., 1988; Savio et al., 1990; Sirois and Fortune, 1988), as well as later studies (Alvarez et al., 2000; Townson et al., 2002; Sartori et al., 2004), reported

Table 2. Total number of follicles ≥ 4 mm diameter on the right, left or both ovaries in purebred Boran and Boran×Holstein crossbred 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	549	9.14±0.091 (Range=4-17)	9.20±0.083 (Range=5-14)	18.34±0.145[a] (Range=11-28)
Boran×Holstein Friesian cross	488	8.69±0.089 (Range=5-17)	8.87±0.084 (Range=4-14)	17.56±0.141[b] (Range=10-28)
Total	1037	8.93±0.064 (Range=4-17)	9.05±0.059 (Range=4-14)	17.98±0.102 (Range=10-28)

[a,b]Means within a column with unlike superscripts are different (P<0.001).

Table 3. Diameter of the dominant, largest subordinate and second largest subordinate ovarian follicle and total number of ovarian follicles in the right and left ovaries in purebred Boran and Boran×Holstein crossbred cows.

Breed	Ovary	N	Mean	SD
	Dominant follicle			
Boran	Right	16	16.19	3.103
	Left	2	17.00	2.828
	Total	18	16.28	3.006
Boran×Holstein cross	Right	10	17.50	1.354
	Left	6	18.17	0.753
	Total	16	17.75	1.183
Total	Right	26	16.69	2.619
	Left	8	17.88	1.356
	Total	34	16.97	2.418
	Largest subordinate follicle			
Boran	Right	16	9.19	2.198
	Left	2	9.00	1.414
	Total	18	9.17	2.093
Boran×Holstein cross	Right	10	8.90	1.287
	Left	6	8.83	1.329
	Total	16	8.88	1.258
Total	Right	26	9.08	1.875
	Left	8	8.88	1.246
	Total	34	9.03	1.732
	Second largest subordinate follicle			
Boran	Right	16	7.38	1.310
	Left	2	7.50	0.707
	Total	18	7.39	1.243
Boran×Holstein cross	Right	10	7.50	0.972
	Left	6	7.33	0.816
	Total	16	7.44	0.892
Total	Right	26	7.42	1.172
	Left	8	7.38	0.744
	Total	34	7.41	1.076

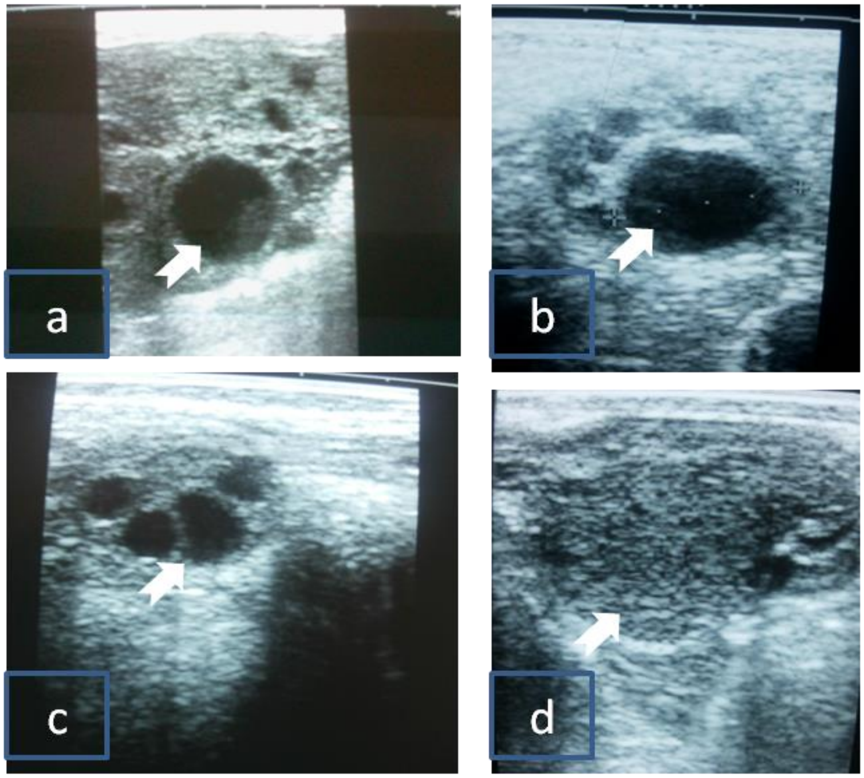

Plate 1. Ultrasonographic images of ovarian structures in purebred Boran cows: Panel a illustrates a dominant follicle; panel b shows several small ovarian follicles plus a large ovarian follicle; panel c depicts medium and large ovarian follicles; panel d illustrates a mature corpus luteum.

that cows with two follicular waves per estrous cycle had a shorter IOI than cows with three follicular waves. Our study revealed the same is true with purebred Boran and crossbred B×H cows raised in Ethiopia, even though cows in our study had a longer IOI than Boran cows in Kenya (Muraya, 2013). Reasons for the difference between Ethiopian and Kenyan Borans are likely due to genetic drift in the different populations (animals selected to live in different climates) and/or difference in feed resources (which under some circumstances could reduce reproductive performance). Our results are in agreement with those reported for the Gir breed in Brazil (Viana et al., 2000).

The results of our study showed fewer waves of follicular growth in Boran than B×H crossbred cows, and these results are in complete agreement with other research groups that investigated *B. indicus* breeds such as the Nelore (Figueiredo et al., 1997; Mollo et al., 2007), the Gir (Gambini et al., 1998; Viana et al., 2000), or Brahman (Zeitoun et al., 1996; Alvarez et al., 2000). Although the wave patterns in Zebu cows are shown to be influenced by parity (Figueiredo et al., 1997), our study was not sufficiently large to directly assess this effect.

The diameter of the dominant follicle (DF) in Boran cows in our study was equivalent to that reported in *B. taurus* cows by Fortune et al. (1988), Savio et al. (1988), and Ginther et al. (1989). However, the diameter of the DF of Boran cows in our study was greater than that reported in other *B. indicus* breeds by Figueiredo et al. (1997) and Sartorelli et al. (2005). The follicular size at the point of deviation in the current study for the Boran is much higher than that reported for *B. taurus* breeds (Ginther et al., 1996; Sartori et al., 2001) and for Zebu cattle (Figueiredo et al., 1997; Sartorelli et al., 2005; Castilho et al., 2007; Gimenes et al., 2008). There is no definitive explanation for this difference. Follicular deviation is characterized by a decrease in the growth rate of the largest subordinate follicle and an increase in the growth rate of the DF (Ginther et al., 2001), and this phenomenon was observed quite clear in purebred Boran as well as B×H crossbred cows.

The total number of follicles greater than 4 mm in diameter in Boran cows was comparable to the number of follicles reported in *B. taurus* breeds (Ginther et al., 1996; Alvarez et al., 2000; Carvalho et al., 2008; Bastos et al., 2010) and lower than the number of follicles reported for Nelore (Buratini Jr et al., 2000; Carvalho et al., 2008; Gimenes et al., 2009; Bastos et al., 2010), as well as Brahman and Senepol (Alvarez et al., 2000). Differences between our results and those of other studies are likely due to differences in climate and available feed resources. Because Boran originated from an area where feed resources typically are not abundant on a year-round basis, perhaps, it developed the ability to grow large numbers of ovarian follicles on a restricted plane of nutrition.

Theoretically, one would expect the function of the left and right ovaries to be comparable. However, in a number of species, including the bovine, the number of ovarian follicles present on (and/or the number of ovulations from) the right ovary typically exceed that of the left ovary by a few percent (Giraldo et al., 2010). Our observation of the right ovaries being more active than the left ovaries in Boran and B×H cows confirms reports of others. It is also in complete agreement with Muraya (2013) who stated that “higher incidence of ovulatory DFs in the right ovary is attributed to the fact that the right ovary receives more blood supply compared to the left one and it is clinically observed to be more active than the right ovary”.

Conclusion

This was the first detailed study of ovarian follicular dynamics of the Boran breed of cattle in Ethiopia. The inter-ovulatory interval of the Boran lies in the range reported for other zebu breeds. However, unlike other zebu breeds where the maximum size of the dominant follicle is typically smaller than that of *B. indicus* cows (Sartori and Barros, 2011), the maximum diameter of the preovulatory dominant follicles were larger and similar to that seen in F_1 crossbred B×H cows. The uniformity of two waves of follicular growth per estrous cycle in the Boran would likely permit more predictable and efficacious application of reproductive biotechnologies such as synchronization of estrus or embryo transfer (Degefa et al., 2016). In addition, the greater number of growing ovarian follicles in the Boran would be ideal for ovum pick-up and subsequent *in vitro* embryo production. Great opportunity exists to capitalize on the reproductive potential of the Boran, and further investigations on advanced reproductive biotechnologies (especially embryo transfer and *in vitro* embryo production) are warranted. It is important to develop effective and sustainable genetic improvement schemes for this indigenous cattle breed of Ethiopia and to ensure the availability of this breed for future use through creation of an embryo cryobank.

Conflict of Interests

The authors have not declared any conflict of interests.

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