Effect of follicular ablation and gonadotropin priming on the recovery and quality of oocytes in Boran cows

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Received: 13 October 2021 / Accepted: 31 August 2022 © The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
The objective of the study was to evaluate the effect of follicle ablation, exogenous FSH application, and different coasting time prior to ovum pick-up (OPU) on the number of follicles suitable for aspiration, oocyte quality, and cleavage rate in Ethiopian Boran cows. The experiment was carried out in three parts, (I) cows were synchronized using 500 µg PGF2α given 11 days apart. Cows were then subjected to a biweekly ovum pickup session before ovulation (n = 5) or starting day 7 after ovulation (n = 4) for 3 weeks. (II) Cows were synchronized, and all visible follicles were ablated on the first days of overt estrus, and cows were grouped into those that received a divided dose of 350 IU FSH (n = 5) or 175 IU FSH (n = 5) over 3 days. In both groups, OPU was carried out weekly starting 48 h after the last FSH for 6 weeks. (III) Protocol was similar to part II, but in group with 350 IU FSH (n = 5), coasting period was increased to 72 h. The covariates of follicles and oocyte were not affected (P > 0.05) by corpus luteum presence at OPU. The mean number of medium (7.36 ± 0.57) and large (8.28 ± 0.96) follicles were significantly higher (P < 0.05) in the group that received divided 350 IU FSH. Similarly, the mean number of grade-1 (4.19 ± 0.24) and grade-2 (4.32 ± .27) oocytes, maturation rate (70.41%), and cleavage rate (47.5%) were significantly higher (P < 0.05) in the group that received 350 IU FSH. COC quality was significantly (P < 0.05) influenced by coasting period. However, both maturation and cleavage rates were not affected by the coasting period. This study demonstrated that follicular ablation and treatment with FSH improves follicular population and oocyte recovery rate in Boran cows.

Keywords Boran · Bovine-oocyte · Cumulus oocyte complex · Coasting time · Follicle stimulating hormone

Introduction
Boran cattle are the most suitable types of cattle breed for arid and semi-arid regions of East Africa including Ethiopia due to their adaptive characteristics like tolerance to heat, resistance to diseases, and ability to utilize low-quality forage and relatively better production performance (Azage et al. 2009; Mekonnen et al. 2010). Genetic differences have been suggested as a possible cause for variation in responses to exogenous hormones. Studies indicated that Bos taurus and Bos indicus breeds have responded differently to ovarian stimulation, and their follicular growth patterns are different. Reis et al. (2010) indicated that synchronizing follicular wave emergence prior to OPU improves cumulus oocyte complex (COC) quality and blastocysts only in Brangus but not in Nelore (Bos indicus) cattle while Rodriguez et al. (2010) reported that follicular wave synchronization and superstimulation improved COC quality and blastocyst rate in both Brangus and Angus cattle. The beneficial effect of gonadotropins given prior to OPU and similarly the dose of follicle stimulating hormone (FSH) used for ovarian stimulation has not been well studied in Bos indicus. In previous works, Meintijes et al. (1995) used 40 mg FSH; Chasombat et al. (2013) used 100 mg FSH, while Blondin et al. (2002) and Chaubal et al. (2006) each used 200 mg FSH to stimulate the ovaries prior to OPU. The time of FSH withdrawal (coasting period) before OPU has effect on oocyte maturation and blastocyst development (Blondin et al. 2002; Sirard et al. 2006). The work of Nivet et al. (2012) indicated that the optimal coasting time can range from 20
to 92 h. The objective was to evaluate the effect of follicular ablation, FSH priming, and costing time on oocyte recovery rate and oocyte quality in Boran (*Bos indicus*) cows.

**Materials and methods**

**Study site**

The study was conducted in dairy farm owned by College of Veterinary Medicine and Agriculture, Addis Ababa University, which is located in Bishoftu towns at latitude of 8° 45′ N, longitude 38° 59′ E and at elevation of 1885 m above sea level. The last 10 years mean annual rainfall of the town was 866 mm with a bimodal pattern while the mean annual minimum and maximum temperatures was 14 °C and 26 °C, respectively. The mean relative humidity was 61.3% according to National Meteorology Service Agency of Ethiopia, the 2020 agrometeorological report (NMSA, 2020).

**Study animals and experimental design**

Animals used in this study were non-lactating uniparous Boran cows free from reproductive abnormalities. Cows were freely grazing for 6 h daily and provided with 2 kg roughages (grass hay) and 1.5 kg concentrates consisting of wheat bran, oil cakes, and molasses. Water was freely accessed. The study design was a crossover experimental design in which all cows would pass through each experiment after a rest of two inter-ovulatory intervals (washout period) between successive experiments. The experimental groups were experiment I (OPU without FSH priming in cows with and without CL), experiment II (OPU with follicular ablation and with different dose FSH priming), and experiment III (OPU with follicular ablation, FSH priming, and with different coasting period) (summarized in Table 1).

Briefly in experiment I, all cows were estrus synchronized by giving 500 µg of PGF2α (Synchromate®, cloprostenol sodium, Warburg, Germany) at 11 days apart. After the second PGF2α, cows were visually followed for estrus signs, and the ovaries were scanned by ultrasound (Aloka SSD-500, Japan) twice a day. Upon estrus, cows were randomly divided into two. In the first group, the first OPU was started when cows were in estrus but before ovulation (group CL negative, \( n = 5 \)), and OPU sessions were made twice a week for 3 consecutive weeks on each cow (total of 30 OPU sessions). In the second group, cows detected in estrus were confirmed for ovulation and CL development (ultrasound). At day 7 of ovulation (group CL positive, \( n = 4 \)) the first OPU was started, and OPU sessions were made twice a week for 3 consecutive weeks on each cow (total of 24 OPU). In each group, immediately before each OPU, follicles were quantified, size measured, and classified as small (3–4 mm), medium (5–9 mm), and large (> 9 mm) (Walters et al. 2002). In experiment II, cows were estrus synchronized as in experiment I and when cows were in estrus, all visible follicles were ablated by ultrasound before ovulation, and cows were randomly assigned into either multiple FSH and OPU48 (\( n = 5 \)) or single FSH and OPU48 (\( n = 5 \)). In multiple FSH and OPU48 group, on day 1 (24 h after follicular ablation), day 2, and day 3, respectively, 175 IU, 105 IU, and 70 IU FSH (FOLLTROPIN®, pFSH 141–431 Vetoquinol CANADA) were given divided into morning and afternoon at 12 h (total dose 350 IU FSH). On day 5 (48 h of the last FSH), the first OPU was performed, and the subsequent OPUs were performed weekly in a similar protocol for 6 consecutive weeks. In single FSH group, FSH was given as a single dose (350 IU, IM) to each cow 48 h prior to OPU. Six OPUs were made per week per cow under a similar treatment. All cows were waited to pass 2 interovulatory intervals before being transferred to subsequent treatment. In experiment III, cows were divided into two groups as multiple 350 IU FSH and OPU72 (\( n = 5 \)) and multiple 175 IU FSH and OPU48 (\( n = 5 \)). In multiple 350 IU FSH and OPU72, the treatment is exactly the same as multiple FSH and OPU48 except all OPU sessions were performed at 72 h of the last FSH treatment (72 h coothing period). In multiple 175 IU

| Experiment | Number | Group | Ablation | FSH dosage | OPU start | OPU frequency | Length of OPU |
|------------|--------|-------|----------|-------------|-----------|---------------|---------------|
| I          | 9      | 1; \( n = 5 \) | No       | No          | Before ovulation | 2×/wk        | 3 weeks       |
|            |        | 2; \( n = 4 \) |          |             | 7 days after ovulation | 2×/wk        | 3 weeks       |
| II         | 10     | 1; \( n = 5 \) | All visible follicles | 350 IU, divided | 48 h after last FSH | 1×/wk        | 6 weeks       |
|            |        | 2; \( n = 5 \) | All visible follicles | 350 IU, single | 48 h after last FSH | 1×/wk        | 6 weeks       |
| III\(^a\)  | 10     | 1; \( n = 5 \) | All visible follicles | 350 IU, divided | 72 h after last FSH | 1×/wk        | 6 weeks       |
|            |        | 2; \( n = 5 \) | All visible follicles | 175 IU, divided | 48 h after last FSH | 1×/wk        | 6 weeks       |

\(^a\)Results from group III were compared with the 350 IU, divided FSH and 48 h coothing period (from group II and not with each other in group III)

\( n \) = Number of cows used

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Table 1: Tabular description of the experimental groups

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FSH group, on day 1, day 2, and day 3 of follicle ablation, the cows received 70 IU, 70 IU, and 35 IU FSH, respectively. The FSH was given divided into morning and afternoon. All OPU sessions were performed at 48 h of the last FSH treatment weekly for 6 consecutive weeks.

**Follicular aspiration**

Before follicular aspiration, both ovaries were scanned, and the follicles were counted and categorized into small (3 to 4 mm), medium (5 to 9 mm), and large (> 9 mm) (Walters et al. 2002). A transvaginal ultrasound (Aloka SSD-500, Japan)-guided follicular aspiration was performed using a 55-mm-long 18-gauge stainless steel needle that was attached to a tubing and to the vacuum pump maintained at 80 mm of Hg. Lidocaine 2% (JEIL pharma.co. LTD, Korea) was used as epidural anesthesia to facilitate the handling of the ovaries through the rectum. All follicles of ≥ 3 mm were aspirated into sterile 50-ml conical tube that contained 15 ml of TCM199 medium with HEPES buffer, 10% fetal calf serum, penicillin (10,000 IU/ml), streptomycin (50 µg/ml), and 25 µg/ml heparin.

**Oocyte collection and grading**

Oocytes were graded morphologically based on cumulus cells layers and homogeneity of cytoplasm as follows: grade 1 (G1), three or more compact layers of cumulus oocyte cells and homogenous cytoplasm; grade 2 (G2), two compact layers of cumulus oocyte cells and homogenous cytoplasm, grade 3 (G3), irregular cumulus cells with one layer and dark agglomeration in the cytoplasm or absence of cumulus cell layers and irregular dark cytoplasm (Alves et al, 2014).

**Oocyte maturation, fertilization, and culture**

Oocytes were washed 2–3 times in Tyrodes lactate (TL-HEPES) oocyte wash medium. G1 and G2 oocytes were transferred into TCM199 supplemented by 10% fetal calf serum (FCS), 5 µg/ml bFSH, 50 µg/ml bLH, penicillin G (50 IU/ml), 50 µg/ml streptomycin, and 22 µg/ml sodium pyruvate (Vieira et al. 2014). Oocytes were incubated for 24 h in 500 µL maturation medium covered with mineral oil. The incubator was maintained at 5% CO₂ in humidified air (90–95% relative humidity) at 39.5 °C. After IVM, cumulus cell expansion and extrusion of the first polar body were recorded. The matured oocytes were washed twice in TALP-wash and transferred to TL-Fert (TALP-Fertilization) medium with 10 µg/ml heparin, 22 µg/ml sodium pyruvate, 50 µg/ml streptomycin, 10,000 IU penicillin, and 6 mg/ml fatty acid-free BSA. Semen straw was thawed; the sperms were washed in TL-HEPES (sperm wash) and separated using a Percoll gradient. The final concentration of 1 × 10⁶/mL sperm was used for fertilization. Oocytes and spermatozoa were co-incubated for 24 h at 39.5 °C under 5% CO₂ and 95% humidity. Twenty-four hours after fertilization, the presumptive zygotes were repeatedly pipetted in wash medium (TL-HEPES) and finally cultured in synthetic SOF medium under oil for 24 h at 39.5 °C under 5% CO₂. Every 24 h, the culture medium was changed, and the morulae were physically evaluated based on number and compactness of cell and area of perivitelline space.

**Statistical analysis**

STATA (version 12) is used to analyze the data. The outcome or response variables were number of follicles of different categories, and number of oocytes recovered, oocyte quality, oocyte maturation, and oocyte cleavage while treatment type was independent variable. Means (± SE) were used to compare the response variables. To compare the difference between treatment mean, either t test or ANOVA was used based on the nature of data to be compared. Differences at P < 0.05 were taken as statistically significant.

**Results**

**Follicular and oocyte parameters in presence or absence of corpus luteum**

The details of number of follicles aspirated and oocyte yield are described in Table 2. The mean follicles aspirated and oocytes recovered were not affected (P > 0.05) by CL presence at OPU. Similarly, there was no significant (P > 0.05) difference in oocyte recovery rate, oocyte quality (grades), and oocyte maturation and cleavage rate by CL presence on ovary at OPU (Table 3).

**Table 2 Follicle number aspirated, oocyte yield, and oocyte quality grade by CL presence**

| Item measured                        | Corpus luteum status |
|--------------------------------------|----------------------|
|                                      | Absent   | Present  |
| Total follicles aspirated            | 331      | 325      |
| Mean aspirated follicle (per session) | 10.39 ± 0.7 | 9.44 ± 0.64 |
| Mean follicle by size/per session     |          |          |
| Small (3–4 mm)                       | 4.51 ± 0.43 | 3.41 ± 0.23 |
| Medium (5–9 mm)                      | 3.56 ± 0.29 | 3.59 ± 0.31 |
| Large (> 9 mm)                       | 2.89 ± 0.22 | 2.53 ± 0.24 |
| Total oocytes recovered              | 218      | 211      |
| Recovery rate                        | 65.86%   | 64.92%   |
| Mean oocyte by COC quality grades    |          |          |
| Grade 1 oocyte                       | 2.35 ± 0.21 | 2.54 ± 0.21 |
| Grade 2 oocyte                       | 2.89 ± 0.33 | 3.34 ± 0.33 |
| Grade 3 oocyte                       | 1.72 ± 0.16 | 1.80 ± 0.12 |
Effect of FSH dose and FSH frequency on follicles and oocyte parameters

The details of the mean follicles aspirated per OPU session are indicated in Table 4. The mean (± SE) number of medium and large follicles aspirated was significantly higher (P<0.05) in divided 350 IU FSH than divided 175 IU FSH doses. Comparatively, cows that received 175 IU divided FSH dose had a significantly larger number of medium and large follicles than the control group (Table 4). All sizes of follicular population significantly increased (P<0.05) when FSH priming was given in divided dosage and when the coasting period was 48 h than 72 h.

In the 350 IU FSH, the overall oocyte recovery rate was 63.76 (593/930) and 61.24% (564/921), respectively, at 48 h OPU and at 72 h OPU, and these recovery rates were significantly higher (P<0.05) than the 54.51% (278/510) recovery rate in the 350 IU FSH given as a single dose.

The details of oocyte quality grade and oocyte recovery rate by FSH protocols are indicated in Table 5. The mean grade 1 and grade 2 oocytes were significantly higher (P<0.05) in the 350 IU FSH divided dose than 350 IU FSH given as a single dose. Similarly, the 350 IU FSH divided dose significantly increased mean grade 1 and grade 2 oocytes than the 175 IU divided FSH dose. The 48-h coasting period significantly increased (P<0.05) the mean grade 1 and grade 2 oocytes than the 72-h coasting periods.

The details of mean oocytes cultured, oocytes matured, and cleaved are indicated in Table 6. Maturation rate and cleavage rate were significantly higher when the 350 IU FSH priming was given in divided doses regardless of the coasting time. Similarly, the 350 IU divided FSH dose resulted in significantly higher (P<0.05) oocytes matured both at coasting period of 48 h (3.96 ± 0.49) and 72 h (3.61 ± 0.49) than the 300 IU single FSH dose and 175 IU divided FSH dose. Oocyte cleavage rate and the mean oocytes cleaved were significantly higher (P<0.05) in the 350 IU divided FSH dose than other protocols.

### Table 3

| Item measured              | Corpus luteum status | Absent | Present |
|----------------------------|----------------------|--------|---------|
| Total oocytes cultured*    |                      | 166    | 154     |
| Mean No of cultured oocyte/session |          | 4.17±0.59 | 3.29±0.46 |
| Total no. of oocytes matured |                     | 89     | 74      |
| Mean no. of oocytes matured/session† |          | 2.8±0.54  | 2.13±0.29 |
| Oocyte maturation rate (%) |                      | 53.61  | 48.05   |
| Cleavage rate (%)          |                      | 32.53  |         |
| Mean cleaved embryos/session|                     | 2.16±0.60 | 1.5±0.28 |

* Grade 1 and Grade 2 oocytes were cultured, †oocytes with first polar body and expanded cumulus cells

### Table 4

| FSH dose | FSH protocol and OPU time | Mean (± SE) aspirated follicles by follicle category |
|----------|---------------------------|--------------------------------------------------|
| 350 IU FSH | mFSH/48 h OPU | Small follicle (3–4 mm) 4.30 ± 0.57a | Medium follicle (5–9 mm) 7.36 ± 0.57a | Large follicle (>9 mm) 8.28 ± 0.96a |
| 350 IU FSH | sFSH/48 h OPU | 3.67 ± 0.42b | 3.55 ± 0.28b | 2.88 ± 0.22b |
| 350 IU FSH | mFSH/72 h OPU | 3.72 ± 0.20b | 5.94 ± 0.43c | 6.81 ± 0.55c |
| 175 IU FSH | mFSH/48 h OPU | 3.30 ± 0.17b | 5.02 ± 0.16c | 5.74 ± 0.22c |
| No FSH Control | 4.51 ± 0.43a | 3.56 ± 0.29b | 2.89 ± 0.22b |

a,b,c = Within the column, values with different lower case letters differ significantly (P<0.05)

### Table 5

| FSH dose | Protocol | G1 oocyte | G2 oocyte | G3 oocyte | Total oocyte | Total follicle | Recovery rate |
|----------|----------|-----------|-----------|-----------|--------------|---------------|--------------|
| 350 IU FSH | mFSH/48 h OPU | 4.19±0.24a | 4.32±0.27a | 1.32±0.022a | 559a | 930a | 63.76% |
| 350 IU FSH | sFSH/48 h OPU | 2.95±0.24b | 2.34±0.38b | 0.31±0.28b | 278b | 510b | 54.5% |
| 350 IU FSH | mFSH/72 h OPU | 3.17±0.31c | 3.29±0.31c | 1.24±0.13a | 564a | 921a | 61.24% |
| 175 IU FSH | mFSH/48 h OPU | 3.06±0.32c | 3.32±0.18c | 0.99±0.43b | 454c | 741c | 61.28% |
| NoFSH Control | 2.35±0.21b | 2.89±0.33b | 1.72±0.16a | 218ab | 331ab | 65.86% |

a,b,c = Within columns, cells with different lowercase letters differ significantly (P<0.05)

G = grade of oocyte
In present study, mean aspirated follicles, recovered oocytes, COC quality grade, and mean numbers of matured and cleaved oocytes were not affected by CL presence on the ovary. The probable reason CL did not negatively affect oocyte recovery rate might be the progesterone released by CL results in the regression of dominant follicles and emergence of new follicle-wave which would increase uniform follicles with competent oocytes. Progesterone may also allow follicles to be exposed for a longer period to low amplitude of LH pulse resulting in constant turnover and also allow follicles to be exposed for a longer period to low amplitude of LH pulse resulting in better quality oocytes and thus improve oocyte recovery rate. Penitente-Filho et al. (2015). Consistent to the present finding, Takuma et al. (2007) and Sugulle et al. (2008) reported that the presence of CL did not negatively influence cleavage rate and blastocyst development. Other factors like stage of the estrus cycle and ovarian condition at oocyte aspiration may contribute to variation in CL effect on oocyte recovery rate and quality in previous works (Snijders et al. 2000; Wolfenson et al. 2000; Neglia et al. 2003; Sugulle et al. 2008). Shabankareh et al. (2013) and Penitente et al. (2015) reported that follicular fluid biochemical metabolite concentration was related to the presence or absence of corpus luteum.

In the present study, FSH administered in divided dose significantly improved mean medium and large follicles at OPU, mean number of oocytes recovered, and oocyte quality. Exogenous FSH injection results in transient increase in serum FSH which will promote the emergence of new small follicles and their growth. The increase in number and size of the ovarian follicles makes them accessible to OPU and finally result in better oocyte recovery rate in the FSH-treated group. The increase in serum FSH may also delay the atresia of subordinate follicles, may increase their size and make them accessible to OPU (Mihm et al. 1997). Similar to the present finding, Lonergan et al. (1994) reported that administration of six injections of pFSH beginning 3 days prior to slaughter resulted in a significant increase in the proportion of follicles > 6 mm in diameter compared to that in non-treated controls. Some other previous works (Goodhand et al. 2000; De Roover et al. 2005; Vieira et al. 2016; Egashira et al. 2019) reported that multiple FSH administration prior to OPU significantly increases the number of follicles aspirated, the number of oocytes recovered, and COC quality than single-dose FSH.

The mean G1 and G2 oocytes (4.19±0.24 Vs 4.32±0.27 respectively) were significantly higher when follicles were aspirated at 48 h coasting period than aspiration at 72 h costing period (3.17±0.3 Vs 3.29±0.31). This may be due to that prolonging the costing period to 72 h may produce follicular atresia, induce atresia of cumulus cells, and decrease oocyte quality. It may also be due to that at 48 h coasting, the follicles are smaller and have smaller follicular fluid than follicles at 72 h which is likely associated with lower intra-follicular pressure that may favor oocytes. Seneda et al. (2005) reported that the efficiency of oocyte recovery was greater when follicles were aspirated at 48 h (2 days) of FSH (at small follicles) than when follicles were aspirated on day 5 of estrus (at large follicles).

In the present study, divided FSH administration prior to OPU significantly (P < 0.05) increased the mean number of matured and cleaved oocytes. The increase in mean number of matured and cleaved oocytes in this finding was probably due to that the divided FSH dose increased overall oocyte recovery rate and grade 1 and grade 2 oocytes. The effect of FSH on oocyte cleavage rate differs from study to study and remains inconsistent (Lopes et al. 2006; Egashira et al. 2019), and the source for the difference might be due to cattle breed used and/or the physiologic status of the cows used. It could be concluded that in Boran cows, follicular ablation and treatment of cows with FSH prior to aspiration improve follicular population and oocyte recovery rate during ovum pickup. The 350 IU FSH given in divided doses and OPU after 48 h coasting period were effective for higher oocyte recovery rate than the 175 IU FSH given in divided doses. The 175 IU FSH given in divided doses after 48 h coasting period was effective for higher oocyte recovery rate than with no FSH treatment.
Acknowledgements The authors would like to thank Mr Abiy Shimelis, Mr Seid Ali, and Mr Getachew Deresu who helped us during oocyte collection.

Author contribution Tilaye Demissie generated the research idea (conceptualization), developed the proposal, and prepared the first draft of the manuscript. Tilaye Demissie conducted the research and generated the data. Tefera Yilma, Tamrat Degefa, Gemechu Wirtu, and Alemayehu Lemma have approved the study methodology and edited the first draft and the final manuscript. All the authors have read and approved the manuscript for publication.

Funding This research received small seed grant from Addis Ababa University Research and Technology Transfer under the grant number RD/LT/PY-156/2019 for purchase of hormones and other consumables.

Data availability The corresponding author would give data on a reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Certificate was received (certificate No VM/ERC/25/01/12/2020) from animal research ethics review committee of College of Veterinary Medicine and Agriculture and all procedures was in comply with ERC procedure.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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