

Research Article

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

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Abstract

Genetic differences have been suggested as a possible cause for variation in responses to exogenous hormones. Here we evaluated the effect of follicle ablation, exogenous FSH and 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 500µg 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 three weeks. II) Cows were similarly synchronized and all visible follicles were ablated on the first days of overt estrus which were then further grouped into cows that received a divided dose of 350IU FSH (n=5) or 175IU FSH (n=5) over three days. In both groups OPU was carried out weekly starting 48h after the last FSH for six weeks. III) A similar protocol as in part II was carried out but coasting period was increased to 72hrs for cows that received 350IU FSH as divided dose (n=5) and 48hrs coasting period for single 350IU FSH dose (n=5). 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 group that received divided 350IU FSH. Similarly, the mean number of Grade-1 (4.19±0.24) and Grade-2 (4.32±.27) COC, maturation rate (70.41%) and cleavage rate (47.5%) were significantly higher (P<0.05) in group that received 350IU 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.

Introduction

Boran cattle are the most suitable types of cattle breed for arid and semi-arid regions of East Africa including in 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 [1,2]. The large majorities of crossbreeding in Ethiopia use crossing of Boran with Holstein. Genetic differences have been suggested as a possible cause for variation in responses to exogenous hormones. Moreover, different studies indicated that *Bos taurus* and *Bos indicus* breed have responded differently to ovarian stimulation and follicular growth. Reis, et al. [3] indicate

that synchronizing follicular wave emergence prior to OPU improves COC quality and blastocysts only in Brangus but not in Nelore (*Bos indicus*) cattle while Rodriguez, et al. [4] reported that follicular wave synchronization and superstimulation improved COC quality and blastocyst rate in both Brangus and Angus cattle. The beneficial effect of gonadotrophins given prior to OPU and similarly the dose of Follicle Stimulating Hormone (FSH) used for ovarian stimulation have not been well studied in *Bos indicus*. In previous works, Mentigens, et al. [5] used 40mg FSH; Chasombat, et al. [6] used 100mg FSH, while Blondin, et al. [7] and Chaubal, et al. [8] each used 200mg 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 [7,9]. The work of Nivet, *et al.* [10] indicated that the optimal coasting time can range from 20 to 92h. We evaluated the effect of two FSH doses, FSH given either divided or at one time, and FSH priming at two coasting time on recovery and oocyte quality during OPU in Boran (*Bos indicus*) cows.

Materials and methods

Study animals and experimental design

The study animals were Boran cows with a mean (\pm SEM) body condition score of 4.37 ± 0.74 (on a scale of 1–5, 1=emaciated, 5= obese) and parity one. The study design was a crossover experimental design in which all cows would pass through each experiment after a rest of two inter-ovulatory interval (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 μ g of PGF $_{2\alpha}$ (Synchromate®, cloprostenol sodium, Warburg, Germany) at 11 days apart. After the second PGF $_{2\alpha}$, cows were visually followed for estrus signs and 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 (3–4mm), medium (5–9mm) and large (>9mm) (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 (FA) before ovulation and cows were randomly assigned into either Multiple FSH & OPU48 (n=5) or single FSH & OPU48 (n=5). In multiple FSH & OPU48 group, on day 1 (24h after FA), day 2 and day 3, respectively 175IU, 105IU, and 70 IU FSH (FOLLTROPIN®, pFSH 141- 431 Vetoquinol CANADA) was given divided into morning and afternoon at 12hr (total dose 350IU). On day 5 (48hr of the last FSH), the first OPU was performed and the subsequent OPUs were performed weekly in a similar protocol for six consecutive weeks. In single FSH

group, FSH was given as a single dose (350IU, IM) to each cow 48h prior to OPU. Six OPU were made per week per cow under a similar treatment. All cows were waited to pass through 2 estrus cycles before transferred to subsequent treatment. In experiment III, cows were divided into two groups as multiple 350IU FSH & OPU72 (n=5) and multiple 175IU FSH & OPU48 (n=5). In multiple 350IU FSH & OPU72, the treatment is exactly same as multiple FSH & OPU48 except all OPU sessions were performed at 72hr of the last FSH treatment (72hr coasting period). In multiple 175IU FSH group, on day 1, day 2 and day 3 of follicles removal, respectively cows received 70IU, 70IU and 35IU FSH divided into morning and afternoon (total dose 175IU). All OPU sessions were performed at 48hr of the last FSH treatment weekly for six consecutive weeks.

Follicular aspiration

Before follicular aspiration both ovaries were scanned and follicles were counted and categorized in to small (3 to 4mm), medium (5 to 9mm) and large (>9mm) [11]. A transvaginal ultrasound (Aloka SSD-500, Japan) guided follicular aspiration was performed using a 55mm long 18-gauge stainless steel needle that was attached to a tubing and to the vacuum pump maintained at 80mm 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 50ml conical tube that contains 15ml of TCM199 medium with HEPES buffer, 10% fetal calf serum, penicillin (10,000IU/ml), streptomycin (50 μ g/ml), and 25 μ g/ml heparin.

Oocyte collection and grading

Oocytes were graded morphologically based on cumulus cells layers and homogeneity of cytoplasm as the following: Grade one (G1) three or more compact layers of cumulus oocyte cells and homogenous cytoplasm; Grade two (G2) two compact layers of cumulus oocyte cells and homogenous cytoplasm, Grade three (G3) irregular cumulus cells with one layers and dark agglomeration in the cytoplasm or absence of cumulus cell layers and irregular dark cytoplasm [12].

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 maturation medium prepared from TCM199, 10% fetal calf serum (FCS), 5 μ g/ml bFSH, 50 μ g/ml bLH, penicillin G (50IU/ml), 50 μ g/ml streptomycine, 22 μ g/ml sodium pyruvate [13]. Oocytes were incubated for 24 hrs in 500 μ L maturation medium covered with mineral oil. The incubator

Table 1: Tabular description of the experimental groups.

Experiment	N	Group	Ablation	FSH dosage	OPU start	OPU frequency	Length of OPU
I	9	1; n=5	No	No	Before ovulation	2x/wk	3 weeks
		2; n= 4	No	No	7 days after	2x/wk	3weeks
II	10	1; n=5	All visible follicles	350IU,divided	48hrs after last FSH	1x/wk	6 weeks
		2; n=5	All visible follicles	350IU, Single	48hrs after last FSH	1x/wk	6 weeks
III†	10	1; n=5	All visible follicles	350IU,divided	72hrs after last FSH	1x/wk	6 weeks
		2; n=5	All visible follicles	175IU,divided	48hrs after last FSH	1x/wk	6weeks

† results from group III were compared with the 350IU,dividedFSH and 48hr coasting period (from group II) and not with each other in group III.



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 10µg/ml heparin, 22µg/ml sodium pyruvate, 50µg/ml streptomycin, 10,000 IU penicillin, 6mg/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 1x10⁶/mL sperm was used for fertilization. Oocytes and spermatozoa were co-incubated for 24h 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 24h at 39.5°C under 5% CO₂. Every twenty four hours 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 was 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), oocyte maturation and cleavage rate by CL presence on ovary at OPU (Table 3).

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 were significantly higher (P<0.05) in divided 350IU FSH than divided 175IU FSH doses. Comparatively, cows that received 175IU 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 48hrs than 72h.

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

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

Item measured	Corpus luteum status	
	Absent	Present
Total Follicle Aspirated	331	325
Mean aspirated follicle (Per session)	10.39±.7	9.44±.64
Mean follicle by size / Per session		
Small (3-4mm)	4.51±.43	3.41±.23
Medium (5mm-9mm)	3.56±.29	3.59±.31
Large (>9mm)	2.89±.22	2.53±.24
Total oocytes recovered	218	211
Recovery rate	65.86%	64.92%
Mean oocyte by COC quality grade		
Grade 1 oocyte	2.35±.21	2.54±.21
Grade 2 oocyte	2.89±.33	3.34±.33
Grade 3 oocyte	1.72±.16	1.80±.12

Table 3: Oocyte maturation and cleavage by CL presence or absence.

Item measured	Corpus luteum status	
	Absent	Present
Total oocytes cultured*	166	154
Mean No of cultured oocyte/session	4.17±.59	3.29±.46
Total No of oocytes matured	89	74
Mean No of oocytes matured/session†	2.8±.54	2.13±.29
Oocyte maturation rate (%)	53.61	48.05
Cleavage rate (%)	32.53	30
Mean cleaved embryos/session	2.16±.60	1.5±.28

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

Table 4: Changes in the mean follicles of different category by FSH dose, and by coasting time.

FSH dose	FSH Protocol & OPU time	Mean (±SE) aspirated follicles by follicle category		
		Small Follicle (3-4mm)	Medium Follicle (5-9mm)	Large Follicle (>9mm)
350 IU FSH	mFSH/48h OPU	4.30±0.57 ^a	7.36±0.57 ^a	8.28±0.96 ^a
	sFSH/48h OPU	3.67±0.42 ^b	3.55±0.28 ^b	2.88±0.22 ^b
	mFSH/72h OPU	3.72±.20 ^b	5.94±0.43 ^c	6.81±0.55 ^c
175 IU FSH	mFSH/48h OPU	3.30±.17 ^b	5.02±.16 ^c	5.74±.22 ^c
No FSH	Control*	4.51±.43 ^a	3.56±.29 ^b	2.89±.22 ^b

a, b, c Within the column values with different scripts (letters) differs significantly (P<0.05), * OPU without FSH and without corpus luteum on ovaries was considered as a control

The details of oocyte quality grade and oocyte recovery rate by FSH protocols are indicated in Table 5. The mean grade one and grade two oocytes were significantly higher (P<0.05) in the 350IU FSH divided dose than 350IU FSH given as a single dose. Similarly, the 350 IU FSH divided dose significantly increased mean grade one and grade two oocytes than the 175IU divided FSH dose. The 48h coasting period significantly increased (P<0.05) the mean grade one and grade two oocytes than the 72h 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 350IU divided FSH dose resulted in significantly higher ($P<0.05$) oocytes matured both at coasting period of 48h ($3.96\pm.49$) and 72h ($3.61\pm.49$) than the 300IU single FSH dose and 175IU divided FSH dose. Oocyte cleavage rate and the mean oocytes cleaved were significantly higher ($P<0.05$) in the 350IU divided FSH dose than other protocols.

Discussion

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. In previous work Pirestani, *et al.* [14] indicated that ovaries with CL yield higher average oocyte collected per ovary than ovaries without CL. Findings on the effect of CL presence or absence on aspirated follicles, recovered oocytes and COC quality were inconsistent. The inconsistency among different studies may be due to breed of cattle used, cows' body condition score, and environmental temperature in which cows kept [15-19]. Penitente, *et al.* [20] reported that follicular fluid biochemical metabolites concentration were related to the presence or absence of corpus luteum and this may also be the reason for differences in mean numbers of matured and cleaved oocytes from study to study.

Similar to the present findings, Lonergan, *et al.* [21] 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.* [22]; De Roover, *et al.* [23]; Vieira, *et al.* [24] and Egashira, *et al.* [25] 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 and than no FSH treated control. However, Bols *et al.* [26] and Techakumphu, *et al.* [27] indicated that FSH stimulation facilitates follicle aspiration but it would not improve or even

decrease oocyte recovery rate. Vieira, *et al.* [13] indicated that oocyte recovery rate was significantly lower from FSH treated donors than non-treated. The inconsistency on effect of FSH stimulation on follicles aspirated and oocytes recovered may be due to animal related factors (Age, physiology and estrus cycle stage at OPU) or it may be due to animal management especially of feeding regimen (nutrient), and /or climate in which animals reared. The frequency of OPU made within a week may also contribute to the inconsistency of result in different studies. The works of Lopes, *et al.* [28] and Li, *et al.* [29] indicated viable oocytes and transferable embryos are affected by OPU frequency.

In agreement to present finding, Seneda *et al.* [30] reported that efficiency of oocyte recovery was greater when follicles were aspirated at 48h (2 days) of FSH (at small follicles) than when follicles were aspirated on Day 5 (at large follicles) although the average number of oocytes obtained per cow for each group did not differ. The work of Blondin *et al.* [7] indicated that 48h coasting period gives significantly more 5mm to 10mm follicles than 33h coasting period.

In present study, divided FSH administration prior to OPU significantly ($P>0.05$) increased the mean number of matured and cleaved oocytes and this was in contrast with work of Vieira, *et al.* [24]. In previous works, effect of FSH on oocyte cleavage rate differs from study to study and remains inconsistent [25,28]. The difference in this finding was probably due to the difference in the number of oocytes matured as divided FSH dose increased oocyte recovery rate and oocytes matured and cultured. The source for the difference among these studies might also 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 improves follicular population and oocyte recovery rate during ovum pickup. The 350IU FSH given in divided doses and OPU after 48h coasting period was effective for higher oocyte recovery rate than the 175IU FSH given in divided doses. The 175IU FSH given in divided doses after 48h coasting period was effective for higher oocyte recovery rate than with no FSH treatment.

Table 5: The mean oocyte grade by FSH protocol and by coasting time.

FSH Dose	Protocol	G1oocyte	G2oocyte	G3oocyte	Total oocyte	Total Follicle	Recovery rate
350 IU	mFSH/48h OPU	4.19±0.24 ^a	4.32±.27 ^a	1.32±0.22 ^a	559 ^a	930 ^a	63.76%
	sFSH/48h OPU	2.95±.24 ^b	2.34±.38 ^b	0.31±.28 ^b	278 ^b	510 ^b	54.5%
	mFSH/72h OPU	3.17±.31 ^c	3.29±.31 ^c	1.24±.13 ^a	564 ^a	921 ^a	61.24%
175 IU	mFSH/48h OPU	3.06±.32 ^c	3.32±.18 ^c	0.99±.43 ^b	454 ^c	741 ^c	61.28%
NoFSH	Control	2.35±.21 ^b	2.89±.33 ^b	1.72±.16 ^a	218 ^{ab}	331 ^{ab}	65.86%

a, b, c Within columns cells different scripts (letters) differ significantly ($P<0.05$).

Table 6: The maturation and cleavage rate during different FSH priming and coasting period.

FSH dose	Protocol	Mean Oocytes matured and oocytes cleaved					
		Total oocyte Cultured	Maturation Rate (%)	Cleavage Rate (%)	Mean oocyte Cultured/OPU*	Mean oocyte matured	Mean oocyte cleaved/OPU
300 IU FSH	mFSH/48h OPU	365 ^a	70.41 ^a	47.5 ^a	5.89±.55 ^a	3.96±.49 ^a	2.78±.32 ^a
	sFSH/48h OPU	301 ^c	55.48 ^c	30.1 ^c	3.41±.66 ^b	2.90±.56 ^b	1.77±.32 ^b
	mFSH/72h OPU	339 ^b	67.85 ^a	43.48 ^b	5.06±.56 ^a	3.61±.49 ^a	2.17±.37 ^a
175 IU FSH	mFSH/48h OPU	303 ^c	62.4 ^b	42.33 ^b	4.64±1.11 ^b	2.82±.35 ^b	1.78±.23 ^b
No FSH	Control	166	53.61 ^c	32.53 ^c	4.17±.59 ^b	2.8±.54 ^b	2.16±.60 ^a

a, b, c Within columns cells with different scripts (letters) differ significantly ($P<0.05$), * cultured oocytes were grade one and grade two oocytes.



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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.

Availability of data and material

The corresponding author would give data on a reasonable request

Authors' contributions

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

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