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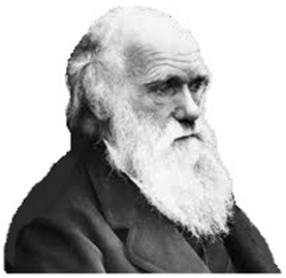

World News of Natural Sciences

An International Scientific Journal

WNOFNS 34 (2021) 88-97 EISSN 2543-5426

Effect of breed, parity and frequency of collection on quality and quantity of OPU derived oocytes

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

[1]Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

[3]National Agricultural Biotechnology Research Center, Ethiopian Institute of Agricultural Research, Holleta, Ethiopia

*E-mail address: sayid1731@gmail.com

ABSTRACT

The aims of this study was to see the effect of breed, parity and scheme of collection on the quality and quantity of OPU derived oocytes and effect of transvaginal ultrasound guided follicular aspiration on subsequent fertility of donor cows. A total of 28 animals (11 Boran and 11 Boran Holstein-Friesian cross for once per week collection, and 7 Boran and 7 Boran Holstein-Friesian cross for twice per week collection) were used in this experiment. A total of 22 animals were evaluated for the effect of the OPU procedure on subsequent reproductive function. In 150 Ovum Pick up sessions, irrespective of collection scheme, breed and parity, 1124 follicle (554 in Boran and 570 in HF crosses) were punctured with a recovery rate of 51.1% in Boran and 48.6% in the crosses. Relatively more follicles were aspirated in crosses compared to Borans although the difference in oocyte recovery rate and quality were not statistically significant. Overall recovery rate was 57.7% in heifers and 42% in cows. Frequency of aspiration significantly influenced ($P<0.05$) oocyte recovery rate with more follicles aspirated in Once per week per session compared to twice per week per session but there was no difference in the mean number of recovered oocytes. A significantly higher number of follicles were aspirated ($P<0.05$) and good quality oocytes were greater in heifers than cows. Different COCs quality grades were recovered from breed, parity and frequency of collection category. Overall from a total of 574 collected COCs, about 326 (57%) of the recovered COCs were of the quality grades I and II, whereas 248 (43%) were with quality grades III and IV. All 22 animals showed estrus on average after 19 days of the last OPU procedure though a relatively weaker intensity of estrus signs, irregularity of the cycle and minor ovarian morphological change were noted. Response rate to a single $PGF_{2\alpha}$ treatment on 18 animals was 72% (13/18). Subsequent insemination of those in estrus resulted in 53.8% (7/13) first service conception

(Received 10 November 2020; Accepted 24 November 2020; Date of Publication 25 November 2020)

rate. In conclusion, parity, and to some extent frequency of collection had a significant effect on the quality and quantity of oocyte but not breed. Animals at post OPU were able to continue cycling and become pregnant.

***Keywords*:** Breed, Ovum Pick, Oocyte quality, Oocyte numbers, Parity, OPU scheme, Crossbreeding Holstein-Friesian, Ethiopian Boran, *Bos taurus*

1. INTRODUCTION

Cattle occupy an integral part in the livestock economy of Ethiopia as well as of the world. Boran, "Indigenous African cattle with potential" 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). Few countries have been able to improve Borans, particularly with the advent of reproductive technologies that played the greatest role in breed improvement (Nandi *et al*., 2000). Ovum Pick Up (OPU), has opened new perspectives by overcoming some disadvantages of superovulation hence it is increasingly used in cattle both as an alternative to superovulation as well as an opportunity to increase the maternal contribution to genetic improvement. OPU success rate is quantified firstly in terms of the oocyte recovery rate (number of COCs per 100 punctured follicles). However, both the numbers and quality of retrieved COCs, is influenced by both technical and biological factors (Merton *et al*., 2003; Niemann and Wrenzycki, 2018).

Bos indicus female normally have more small ovarian follicles than the *Bos taurus* breeds and a few reports indicate higher mean oocyte production per OPU from *B indicus* cows (Pontes *et al*., 2011; Bols *et al*., 1996).

It has been reported that cow ovaries provide lower numbers of oocytes than maiden heifers (Lonergan, 1990; Moreno *et al*., 1992). Studies on the effect of age on blastocyst yield showed higher from animals between 1 to 3 years of age than older animals. A number of different puncture schedules have been tried for OPU. They have involved puncture on different days in the cycle (days 3-4, 9-10, or 15-16 after the day of estrus, Pieterse *et al*. (1991), and different intervals between punctures, with punctures after 48 and 96 h, Simon *et al.* (1993), once weekly, Petyim *et al.* (2000), or twice weekly, Gibbons *et al*. (1994). The twice weekly puncture was mostly done continuously at 2-5 or 3-4 day intervals, Gibbons *et al*. (1994).

The most popular aspiration schedule is once weekly (Looney *et al.*, 1994). However, a more frequent collection schedule may be important for recovering oocyte during the growing phase or for maintaining a constant crop of follicle in subsequent aspiration. Regarding frequency of collection, even though once weekly is the most popular aspiration schedule (Looney *et al.*, 1994), most studies have concluded that twice weekly collection of oocytes provides a higher number and better-quality oocytes for subsequent IVEP (Galli *et al*. 2001; Merton *et al*. 2003; Petyim *et al.* 2003). This is hypothesized because of lack of development of a dominant follicle that induces atresia in the developing follicles (Mihm and Austin, 2002). However, report by Imai *et al.* (2000) shows that OPU performed with 7 days (under once a week) interval by skilled personnel on non-super stimulated animals was more efficient in bovine punctured follicle, recovered oocyte and IVEP than twice weekly aspirations. The authors believe that the finding might be related to breed, parity, lactational status or other external factors affecting the ovarian function.

The ultimate test for oocyte quality is its ability to be fertilized and to develop to the blastocyst stage and finally to establish pregnancy which results in a living offspring (Lonergan *et al.*, 2001). Unfortunately, it is practically impossible to *in vitro* mature, culture, fertilize and transfer all collected oocytes in living recipients to check for pregnancy. Therefore, it is obligatory to use other parameters, which are said to be well correlated with the actual oocyte quality. The most reliable and commonly used parameter in evaluating oocyte quality is the number and morphology of COCs layers surrounding the oocyte and ooplasm (Hawk and Wall, 1994). Therefore the present study was undertaken to evaluate the effect of breed and parity on quality and quantity of OPU derived oocytes and to determine impact of OPU on subsequent donor fertility.

2. MATERIALS AND METHODS

2. 1. Experimental animals and managements

The study animals were indigenous purebred Boran and Boran * Holstein Friesian (HF) Crossbred heifers and cows aged between 2 and 10 years. The average body condition score was between 2.5 and 3.5 (on a 1-5 scale) as described in Wildman *et al*. (1982). Donor animals were fully managed intensively and were provided with feed of different mix: *tef* straw (*Eragrostis tef)* and grass hay (*Andropogon abyssinicus*) as a basal diet and supplemented with commercially prepared concentrate, mineral salts, and alfalfa green fodder. Water was provided *ad-libitum*.

Animals were dewormed for the suspected parasitic disease and vaccinated at regular interval lampy skin disease (LSD), foot and mouth disease (FMD), and other viral diseases. Artificial inseminations were used as breeding tools. Animals were housed in a well-ventilated concrete floor house.

2. 2. Experimental design

A total of 28 animals (14 Boran and 14 Boran * Holstein crosses) were randomly selected and subjected to an OPU session of once and twice weekly (**Table 1**).

Table 1. Design of oocyte collection with breed and parity category using one and twice a week frequency of collection.

	No. of animals (Once per week collection)		**No. of animals (Twice per week collection)**	
Breed	**Heifers**	**Cows**	**Heifers**	**Cows**
Boran	6	5	4*	3
Cross	6	5	4*	3
Total	**12**	**10**	**8**	**6**

* Number of heifers used repeatedly (for twice a week of after a rest of once a week scheme)

OPU was performed on the same day of the week for each group with the objective of keeping a consistent aspiration interval of 7days in the TVFA-once per week group, and alternate intervals of 2 and 5 days in the TVFA-twice per week group for each breed Latica *et al*. (2019).

The animals were restrained in a suitably designed chute (crush) which allowed minimal movement. Prior to follicle aspiration, each cow was given 2-3 ml epidural injection of 2% Lidocaine.

OPU was performed using a 6.5 MHz frequency transvaginal transducer (Aloka SSD-500, GmbH, and Minitube Germany). The collection apparatus consist of 1.2 × 75 mm (for cows) and 0.9 × 70 mm (for heifers) disposable long single lumen needle along stainless steel dorsal needle guidance attached to sterile aspiration line or 2-m long silicon tube fitted to an aspiration pump that has a warming block (mini-tube, GmbH, Germany) adjusted to 38.7 ^{o}C. A vacuum aspiration pressure of 72 mmHg to 80 mmHg equivalent to a flow rate of 15-18 ml/min was connected to a 50 ml falcon tube containing 5 ml Dulbecco's phosphate buffered saline (DPBS) as an oocyte recovery medium.

Oocyte recovery media were prepared from DPBS supplemented with heparin 20 µg/ml, 2% FCS, 50 µg/ml gentamicin, and 25 mM HEPES. After a thorough mixing of the ingredients, the medium was sterilized by filtration using a 0.2 µm sterile low protein binding acrodisc syringe filter and then kept in a CO_2 incubator maintaining 5% CO_2 at 38.5 ^{o}C with 90% - 95% relative humidity.

At each OPU session, the internal genitalia and the ovaries were palpated rectally and ultrasonically identified.

The number of follicles on both ovaries were counted and measured, and the presence or absence of CL and follicular cyst were recorded prior to aspiration. After removal (ablation) of dominant follicle, all the rest visible follicles from both ovaries were aspirated. After aspiration of all visible follicles, an extra 1-2 ml of medium was aspirated to recover oocytes from the needle and silicone tubing. The aspirated fluid was poured on a searching Petri dish placed on warming plate (HT 50; Minitube, Germany) regulated to 37.3 ^{o}C and waiting for 5-10 minutes to settle and searched under the stereo microscope. The COCs recovered were morphologically graded into four categories according to number and morphology of cumulus cell layers cytoplasmic membrane and zona pellucida.

Animals were followed for at least two cycles post OPU. Estrus signs, cyclicity, morphological changes of the ovaries and ovarian structures were used to evaluate the impact of OPU on donor animals. The animals were subjected to estrus synchronization to evaluate response to the treatment. AI was carried out at standing heat in responding animals and pregnancy was checked 30 days post AI.

3. RESULTS

The result of the current study is the first report of its kind in Ethiopia in evaluating the effect of breed, parity and different TVFA schedules on the number of aspirated follicles, number and quality of recovered oocytes performed on pure Boran breeds and its cross with Holstein Friesian. Counting and aspirating ovarian follicles are shown in **Figure 1**, whereas different quality grade of oocytes collected from Boran and Boran*Holstein cross collected by OPU are shown in **Figures 2** and **3**.

Figure 1. Imaging of follicle in OPU (left Panel) ovarian follicle before aspiration (right Panel)

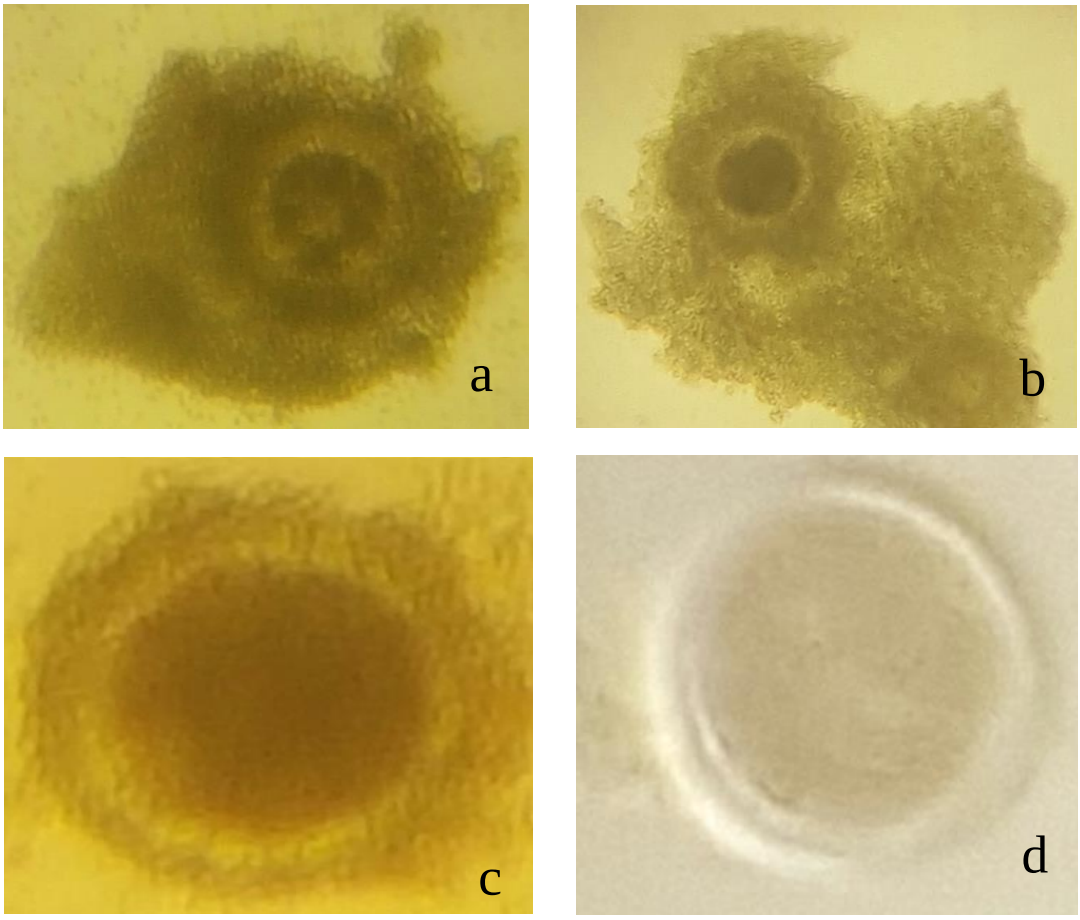

Figure 2. Oocyte recovered from Boran donors using OPU
(**a** = Grade I; **b** = Grade II; **c** = Grade III; **d** = Grade IV)

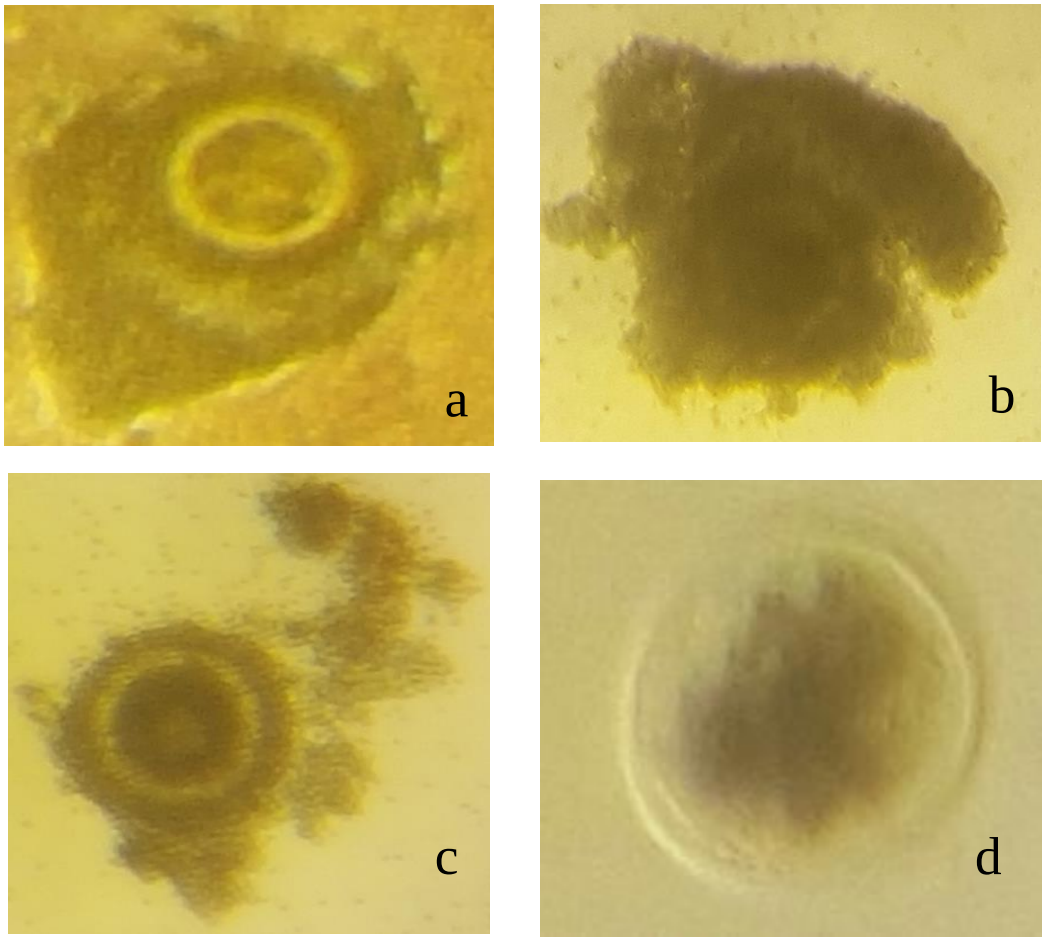

Figure 3. Oocyte recovered from Boran*HF donors using OPU (**a** = Grade I; **b** = Grade II; **c** = Grade III; **d** = Grade IV).

In 150 OPU sessions, irrespective of collection scheme, breed and parity, 1124 follicles (554 in Boran and 570 in HF crosses) were punctured and 574 oocytes were recovered with 51.06% recovery rate (**Table 2**).

Irrespective of breed and collection scheme, in 84 OPU sessions, 685 follicles (8.15 follicles per session) could be observed and 395 oocytes (4.7 oocytes per sessions) were collected from heifers. Out of these, 227 oocytes (57.5%) were with a quality grade I and II, whereas 168 oocytes (42.5%) were with a quality grade III and IV. On the other hand, in a total of 66 sessions, 439 follicles (6.65 follicles per sessions) could be observed on cow's ovaries and 179 oocytes (2.71 oocytes per sessions) were collected from cow ovaries. From these, 99 oocytes (55.30 %) were with a quality grade I and II and 80 oocytes (44.70%) were with a quality grade III and IV.

Irrespective of parity and collection scheme, in 75 sessions 554 follicles (7.38 follicles per session) could be observed and 291 oocytes (3.88 oocytes per sessions) were collected from Boran donors. 170 (58.41%) oocytes were with a quality grade I and II and 121 (41.59%) were with a quality grade III and IV.

On the other hand, for the same sessions 570 follicles (7.60 follicles per session) could be visualized and 283 oocytes (3.77 oocytes per sessions) were collected from Boran crossed Holstein breed donors. From a total of collected oocytes, 156 (55.12%) were with a quality grade I and II and 127 (44.88%) were with a quality grade III and IV.

Table 2. Recovery efficiency of oocytes quality and quantity with different quality with once and twice per week scheme of collection.

OPU scheme	**OPU session**	No aspirated follicle (±SD)	No collected Oocyte (±SD)	Number of COCs quality grade (%)			
				I	**II**	**III**	**IV**
Once/week	66	522 (7.9±1.6)	258 (3.9±1.3)	58 (22.5)	76 (29.5)	76 (29.5)	48 (18.6)
Twice/week	84	602 (7.12±2.4)	316 (3.76±1.8)	96 (30.4)	96 (30.4)	86 (27.2)	38 (12.0)
Total	**150**	**1124**	**574**	**154**	**172**	**162**	**86**

4. DISCUSSION AND CONCLUSSION

It was evident from the results that frequency of aspiration significantly influenced ($P<0.05$) oocyte recovery rate with more follicles aspirated in once/week compared to twice/week but there was no difference in the mean number of recovered oocytes. A significantly higher number of follicles ($P<0.05$) were aspirated and better quality oocytes were recovered from heifers than from cows. Previous reports by Lonergan (1990) and Moreno *et al.* (1992) agree with this finding that cow ovaries provide lower numbers of oocytes than maiden heifers. Again it was in a strong agreement with Rizos *et al.* (2005) reports which showed a higher number of follicles available for puncture on the ovaries of heifers than cows (10.4 versus 7.8, respectively) and a significantly higher number of total oocytes were recovered from heifers than from cows (3.0 versus 1.8), respectively. Similarly greater numbers of good quality and culturable (grade I and II) oocytes were recovered from heifers than cows.

Relatively more follicles were aspirated in crosses compared to Borans although the difference in oocyte recovery rate and quality was not statistically significant. The mean numbers of aspirated follicles in both breeds were lower than earlier reports without FSH treatment: 12.28 in Thai Indigenous Beef Cattle (*Bos indicus*) breed (Sakhong *et al.*, 2012). However, it was greater in mean total harvested oocytes (2.27 per donor and 2.46) in northern Thai native cattle (Apimeteetumrong *et al.*, 2009) treated with FSH; and 2.22 in pregnant Angus breed treated with eCG (Aller *et al.*, 2012).

Ovum pick-up is considered to be a mildly invasive technique employed to retrieve oocytes repeatedly (Bols *et al.*, 1995). A total of 22 animals were evaluated for effect of the OPU procedure on subsequent reproductive function for at least three cycles. All 22 animals showed estrus on average after 19 days of the last OPU procedure though signs were relatively weaker in intensity. Transrectal manual palpation and visual observation showed a minor

morphological change of the ovaries (ovarian hardening), and irregularity of the estrus cycle, respectively, and is in agreement with the previous studies reported by Boni *et al*. (1997) and Petyim *et al*. (2000, 2001). For further refinement, response rate to a single $PGF_{2\alpha}$ estrus synchronization treatment on the 18 animals was 72% (13/18). Subsequent insemination of those in estrus with sex sorted semen resulted in 53.8% (7/13) first service conception rate. In conclusion, parity, and to some extent, frequency of collection had a significant effect on oocyte quantity and quality but not breed. Animals at post OPU are able to continue cycling and become pregnant.

Acknowledgements

The authors gratefully acknowledged Ethiopian Institute of Agricultural Research (EIAR), Debre zeit Agricultural Research Center (DZARC), National Agricultural Biotechnology Research Center (NABRC), Holleta Agricultural Research Center (HARC) and Addis Ababa University College of Veterinary Medicine and Agriculture for providing infrastructure and all the facility for carrying out this study. The assistance of Tariku Bekele, and DZARC animal biotechnology and dairy research program staff is gratefully acknowledged.

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