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In vitro maturation of oocytes retrieved from Boran and Boran Holstein crosses by OPU technique

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Abstract

The choice of maturation and culture media is a very critical element in the success of *in vitro* embryo production. The difference in reproductive physiology between breeds may vary the in the intrinsic quality of oocytes which is essential for developmental competence and can influence the *in vitro* maturation ability of oocytes. In the current study *in vitro* maturation differences of oocytes from Boran and Holstein *Boran crossbred cows obtained through ovum pickup were evaluated. Oocytes with ≥ 3mm diameter aspirated from 288 follicles were matured in BO-IVM and TCM-199 maturation media. The overall maturation rate of oocytes from Boran and crossbreeds was in the order of 79.4% and 79%, respectively, and statistically, no differences between the breeds were observed. However, all maturation indices significantly higher ($p<0.05$) maturation of oocytes was observed in BO-IVM (88.7%) compared to TCM-199 (70%). Maturation of crossbred and Boran oocytes in BO-IVM was (90.3%) and (86.8%) respectively whereas 67.7% and 72.2% for respective breeds in TCM-199. Maturation rate in terms of cumulus cell expansion in BO-IVM (59.3±3.9%) fully expanded and (30.1±4.6%) partially expanded) was relatively better than in TCM-199 (46. 9 ±0.9%) fully expanded and 24.4±4.3% partially expanded). Extrusion of the polar body was evident in (55.6±5.1%) of oocytes matured in BO-IVM as compared to (41.3±6.0%) in the TCM-199 medium. In conclusion, oocyte maturation following OPU was influenced by media type but not by breed.

Keywords: Boran, crossbreed, in vitro, maturation, oocytes

1. Introduction

Transvaginal ultrasound-guided oocyte collection or Ovum pick-up is a common method of recovering oocytes from live animals. This procedure can be used in conjunction with *in vitro* embryo production (IVEP) to improve the number of offspring from genetically valuable cows (Galliet al,.2001)

IVEP is recommended for production of embryos from genetically improved dairy cattle for developing countries, production of hybrid genotypes (i.e.*Bos Taurus*x *Bos indicus*) with the potential for better productive performance especially the tropics. The multiplication and increasing of genetically improved and potentially productive herd can be achieved in a faster way with IVF than with traditional genetics chemes (Galliet al., 2003).

Nevertheless, despite the availability of modern technologies on global market and the huge and genetically diversified cattle population (65.35million heads) resources in Ethiopia, the dismally low outcome of cross-breeding is evident in the very low proportion (1.91%) of hybrids (CSA 2020). Moreover, according to (Degefa et al.,2016a), (Degefa et al.,2016b), (Gadisa et al.,2019) there is a limitation on the availability of information regarding the application of advanced ART techniques on zebu and crossbred dairy cattle at large and specifically on IVEP in Ethiopia.

Oocyte maturation is the most significant stage which influences the subsequent successful fertilization, zygote formation, blastocyst stage, and IVEP. The production of competent oocytes during IVM is important for cattle reproduction concerning the ability to increase the production of valuable, healthy offspring (Rizos et al., 2002). This is because during the stage of COC maturation, oocytes undergo, through several molecular and cellular modifications to acquire developmental competency (Crozet et al., 1995).

The type of maturation media and additives used are the other common factor that affects the developmental competence of bovine oocytes (Ayman et al., 2016). TCM-199 is the most widely used culture medium for bovine oocytes(Thompson, 2000). Similarly, BO-IVM complete medium comprises important substances that enhance oocyte maturation in cattle(Pryor et al., 2016). However, both these media are developed based on the oocytes derived from *Bos taurus*. There is a difference in reproductive physiology between *bos taurus and bos indicus* cows so the intrinsic quality of the oocytes between breeds is also not the same. Therefore, this study hypothesizes that *in vitro* maturation of oocytes would be influenced by breed differences.

2. Materials and Methods

2.1 Study Animal

The study was conducted from December 2020 to June 2021 at Debre Zeit Agricultural Research Center (DZARC) Ethiopia. Totally 10 healthy cows, 5 Borans and 5 Holstein Friesian X Boran cross with 75% exotic blood level and an average body condition ranging 3 to 4 (on the scale of 1-5) and 1 to 2 parity were used for the OPU procedures. All cows were vaccinated for common contagious diseases and managed under a uniform housing system. The study animals were fed on teff straw and grass hey basal diet and supplemented with concentrate (mixture of 50% wheat bran, 25% wheat short, 24% nuge seed cake, and 1% salt) and had ad libitum access to water

2.2 Ovumpick-up(OPU)

Experimental cows were selected from a pool of dairy herds with no history of reproductive diseases and found to be in good health at the time of the experiment. The cows were subjected to gynecological evaluation using ultrasonography to confirm cyclicity as well as the soundness of the reproductive tract. Cows with disorders such as COD or any other identifiable problem were rejected.

Cows had received epidural anesthesia (2 to 5 ml of 2% lidocaine, JEIL.PHARMA.CO, LTD, Daegu, Korea) to prevent straining during aspiration. After emptying the rectum, the vulva and perineal area were thoroughly cleaned and disinfected. OPU was done once weekly and ffollicular aspiration was performed transvaginally on each visible follicle that was ≥ 3 mm in diameter. An ultrasound (Aloka SSD Pro-Sound 2, Japan) with a 6.5 MHz convex array transducer that was fitted into an intravaginal needle guide (Hitachi Medical Co., Tokyo, Japan) was used for visualization of follicles during aspiration. The follicular puncture was performed using a disposable 18-gauge x 12 mm aspiration needle that was connected to a 50-ml conical tube via a 2 m long silicon tube fitted to an aspiration pump that has a warming block (mini-tube, GmbH, Germany) adjusted to 38.7^0C. A vacuum aspiration pressure of 72 to 80 mmHg equivalent to a flow rate of 15-25 ml/min was used for follicular aspiration. Follicles were aspirated into a 50ml tube containing about 10ml of the recovery media supplemented with DPBS 0.9g/100ml, heparin, 2% FCS, gentamicin 0.05gm/100ml, and HEPES 0.024gm/100ml, 2µl Heparin.

2.3 Oocytes searching and selection

Oocytes were searched under a stereo-microscope (Motis SMZ, Roanoke USA). The COCs were examined and selected based on their morphology, the compactness of cumulus, and the homogeneity of the cytoplasm, and graded for maturation according to (Kakkassery et al 2010).

2.4 *In vitro* maturation

Two commercial maturation media were used: the first TCM- 199 base maturation medium ready to use as a 10ml TCM-199stock solution (Gibco Grand Island, NY) into which 0.2ml FSH, 2% FCS, 0.05g Gentamycin, and 022g $NaHCO_3$ were added. The second IVM was BO-IVM (IVF Bioscience,United Kingdom) a complete and optimized medium, used for oocyte maturation.

The selected oocytes were then washed three times in their respective media with TCM-199 maturation media for the TCM group or BO-IVM medium for the BO-IVM group. IVM drops of 500µl were prepared for 20 COCs, in the meantime in a 4-well embryo culture dish and covered with paraffin oil. The oocytes were then placed in a CO_2incubator set at39^ocunder a 90% humidified atmosphere of 5% CO2inairfor 24 h.

2.5 Assessment of maturation parameters of oocytes

COCs expansion and extrusion of first polar body were evaluated under the stereomicroscope after 24 hours of incubation as a key indicators of oocyte maturation. Cumulus cell expansions were rated as indicated below:

Fully expanded COC – more than 5 layers of Cumulus cells spread non- homogenously with no clustered cells.

Partially expanded **–** 3-5 layers of cumulus cells spread homogenously and with the presence of clustered cell.

Unexpanded - the cumulus cells remain attached to the zona.

COCs with fully and partially expanded cumulus cell layers were considered as maturated oocytes.

COCs with polar bodies between zona pellucida and perivitelline space were considered as a COC with first polar body extruded.

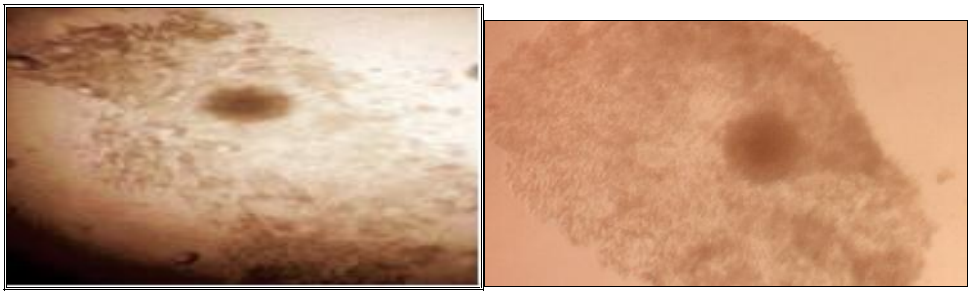
2.6 Statistical analysis

The SPSS version 20 was used to analyze data. Data were grouped according to the breed of the animal and maturation media. Descriptive statistics were used to determine the frequency and proportional maturation rate. The results were considered significantly different at $p < 0.05$.

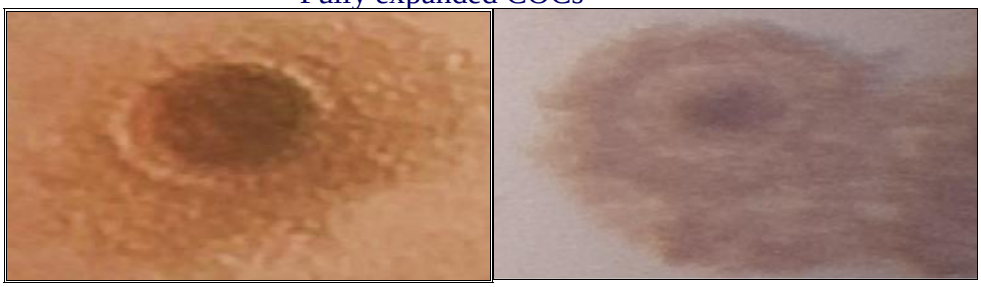
3. Results and Discussion

3.1 Over all maturation rate

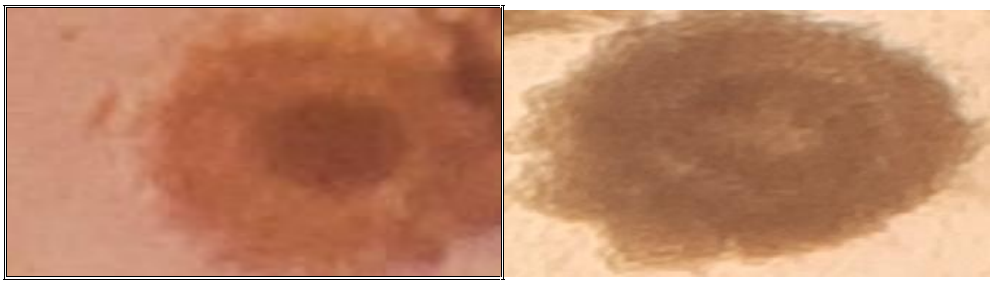
The overall maturation rate of oocytes aspirated from Boran and Crossbred cows was 79.2%, and was comparable with the result of (Islam et al., 1007), in Zebu and (Vijayalakshmi et al., 20202) in Buffalo oocytes. The maturation rate of COCs recovered from Boran and Crossbred were 79.4% (85/107) and 79% (98/124), respectively. The maturation rate in terms of degree of expansion, fully expanded COCs was 52.4% and 54.4% for Boran and Crossbred cows, respectively while 27.2% of Boran and 25.6% of Crossbred COCs were only partially expanded during maturation. The maturation in terms of COC expansion was greater compared to the maturation rate of abattoirs oocytes reported by (Debet al., 2016). First polar body extrusion was recorded in 47.6% of Boran and 49.6% Crossbred cows COCs at the end of maturation were lower than the result reported by(Debet al., 2016), and greater than the reported by (Vijayalakshmi et al., 20202).



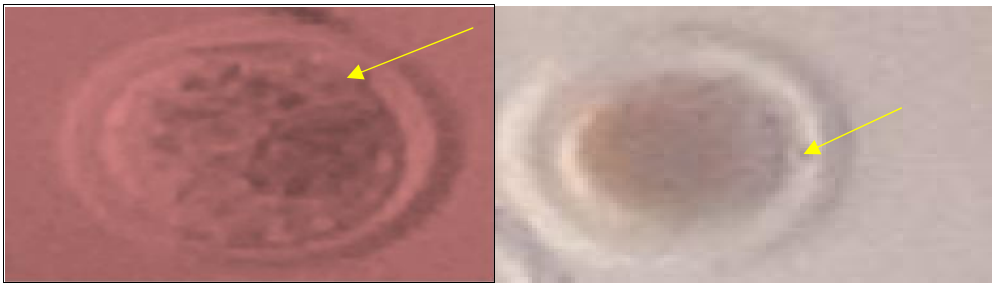
Fully expanded COCs



Partially expanded COCs



Unexpanded COC



COCs with extruded first polar body

Fig 1. Condition of COCs 24 hour post incubation

3.2 Effect of maturation medium on maturation rate

Table 1. Comparison IVM on COCs maturation rate (%± SEM) 24h post incubation

.

Incubated COCs (n)	Media Used	Expanded COCs (n)	COCs expansion (%)	Fully expanded COCs%	Partially expanded COCs	COCs extrude First PB (%)
115	BO-IVM	102	88.7%[a]	59.3±3.9%[a]	30.1±4.6%[a]	55.6±5.1% [a]
116	TCM-199	81	70[b]	46.9±0.9%[b]	24.4±4.3%[b]	41.3±6.0% [b]

Values with different superscripts within the same columns different significantly *(P*< 0.05*)*

The proportion of maturation *in vitro* COCs in BO and TCM-199 media (table 1**)**, was greater than reported by (Rahman et al 2018), (Mohammed et al., 2018), and comparable with the finding of (Sonjaya and Hasbi, 2019).

However comparing between Medias used, the maturation rate was significantly different (P<0.05)in terms of COCs expansion the proportion of COCs (88.7% versus 70%) in BO and TCM-199 maturation media respectively, similarly (55.6±5.1% versus 41.3±6.0 %) of COCs displayed their first polar in BO and TCM-199 maturation media respectively.

It is noticeable that the type of medium and its composition is highly influencing the *in vitro* oocyte maturation. The higher maturation rate of oocytes matured in BO-IVM media may be due to it is complete and optimized media for IVF. In addition to this it contains favorable amount of glucose that conducive for oocyte maturation.(Wrenzycki and Stinshoff, 2013) Described that Glucose is an essential energy substrate when added acceptable amount to IVM, energy is an important requirement for oocyte metabolism. Glucose enhances the resumption ofmeiosis in cattle COCs which is fundamental for oocytes to achieve full developmental competence for fertilization. Glucose also support COCs maturation by converted to extra-cellular matrix component which involved in COCs expansion (Downs et al., 1998).

3.3 Effect of breed on COCs maturation rate

Table 2. Maturation rate of COCs obtained from two different breeds

IVM Media	Breed	Incubated COCs(n)	Expanded COCs %,(n)	**C**OCs extrude First polar body %,(n)	P-value
BO-IVM	Boran	53	86.8% (46)	52.8% (28)	> 0.05
	Cross	62	90.3% (56)	58% (36)	
TCM-199	Boran	54	72.2(39)	44.4% (24)	> 0.05
	Cross	62	67.7(42)	38.7% (24)	

The maturation rate of Boran and crossbred cows' oocytes (table 2) COC expanded cumulus cells and extruded first polar body from was (86.8% versus 90.3%) and (52.8% versus 58%) in BO maturation media respectively, likewise the proportion of matured oocyte from respective breeds exhibited COC expansion and first polar body 24 hour post incubation was (72.2% versus 67.7%) and (44.4% versus 38.7%) in TCM-199 maturation media respectively. There was no significant (p>0.05) effect of breed on maturation rate of COCs mature on the same maturation media. This result point out the oocytes from each breed has comparable potential of maturation rate within same condition.

As so many investigators suggested the efficiency of *in vitro* oocytes maturation relayed on different factors. For instance: the quality and composition media(Alofi and Alhimaidi,2004),quality of Cumulus vestment at the beginning (Ayman et al., 2016), (Sirard et al., 2006),size of follicles at the time of aspiration Fair, (2003), (Lojkić et al., 2016), duration of maturation (Aguila et al., 2020) and incubation temperature (Şen and Kuran, 2018) are the most important factors rather than breed difference.

4. Conclusion

Media is one of the vital and determining factors in vitro embryo production particularly the *in vitro* maturation of COCs. The COCs matured in the media employed for this study BO-IVM, were best in maturation rate than the oocytes matured in TCM-199. Thus, it can be concluded that oocyte maturation following transvaginal oocyte aspirations was influenced by employed media type. In general, the result indicated the impact of media compositions on in vitro embryo production procedures. The fact that breed influences are not observed in this study, so that *in vitro* oocyte maturation hints a promising fact for propagation of cross bred animals that are highly needed to meet the demand for replacement heifers in the newly growing commercial dairying.

Although IVEP has a great deal of contribution to breed improvement, laboratory inputs are scarce for laboratories such as those in Ethiopia. Therefore, it is highly recommended that a small scale media optimization work is carried out for indigenous breeds before scaling up.

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