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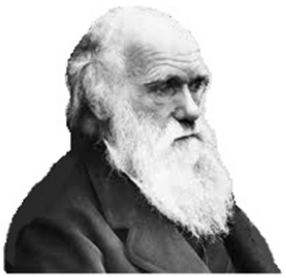

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Presence of CL influence the quantity and quality of COC in slaughter house derived ovaries of Boran heifers

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ABSTRACT

The objective of the study was to determine whether the presence of CL on the ovary can influence the number and quality of COCs recovered using needle aspiration technique. A total 40 numbers of Bovine ovaries were collected from the local abattoirs soon after the animals were slaughtered. The specimens were classified as corpus luteum present (CL+) and corpus luteum absent (CL−) groups. A total of 583 follicles were aspirated from 40 ovaries and among them 381 (62.5%) COCs were collected from CL− group ovaries and 202 (37.5%) COCs from CL+ group ovaries. The COCs were also classified as Grade I (>3 layers of cumulus), Grade II (2 or 3 layers of cumulus), Grade III (no cumulus) and Grade IV (degenerated cells). Grade I and Grade II were classified as normal, and Grade III and Grade IV were considered as abnormal COCs. The result indicated that greater numbers of follicles were aspirated and COCs were collected from CL− ovaries (15.24±5.27 and 9.40±3.50, respectively) than ovary with CL+. Ovaries CL− contribute higher normal COCs (Grades I and II) than that of ovaries with CL+. The result of this study is a preliminary work directing suitable source of COCs for initiating and optimizing in vitro embryo production experiment in like Ethiopia having huge cattle population and animal slaughtering is more common for various reasons like to export or for domestic consumption.

***Keywords*:** Aspiration technique, Boran heifer, ovarian type, Slaughterhouse ovary, *Bos indicus*

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1. INTRODUCTION

The Boran cattle breed is zebu types that originated in the southern lowlands of Ethiopia. The breeds have unique traits that make them suitable for the harsh environment in the lowlands and have ever been part of the pastoralist's identity. It is widely used for milk, meat, draught power and manure production (Albero & Hailemariam, 1982). Pontes *et al*., (2010) report that, *Bos indicus* female normally has more small ovarian follicles than the *Bos taurus* breeds. Boran cattle breed is also the only indigenous breed exposed to assisted reproductive technology other than AI, recently like multiple ovulation and embryo transfer (MOET) and Ovum Pick up (OPU) technique in the process of breed improvement planning with the promising findings in Ethiopia.

The ovaries are the primary organ in a bovine reproductive tract which produce eggs. Follicles and corpus luteum are structures found on the surface of ovary. Just after ovulation, remaining cells of the follicle are initially formed in to corpus hemorrhagicum and then fill the cavity of the ruptured follicle. After that, under the influence of luteinizing hormone, the granulosa cells lining the empty follicular cavity begin to multiply and form a corpus luteum.

Thus, there are two types of ovaries that can be classified which are one with CL (CL+) and other without CL (CL−). Usually, from slaughtered animals higher number of ovaries are obtained, having no CL compared with CL as higher less reproductively performing animals were slaughtered due to economic reason. An experiment done by Khandoker *et al.* (2011) on evaluation of buffalo ovaries, follicles and COCs with the view of IVEP found that the number of observed follicles, aspirated follicles, number of COCs, as well as number of normal COCs were significantly higher in ovaries CL− than CL+.

Each ovary contains thousands of oocytes at birth, most are lost through atresia. *In vitro* embryo production (IVEP) in cattle begins with recovery of oocyte from live or slaughter animals. The oocytes can be recovered from abattoir ovaries in large number which constitute cheapest and economical source of oocytes. This paves the way for large scale and economic embryo production (Sianturi *et al*., 2002). Developmental competence of oocytes to develop into embryo under *in vitro* conditions begins with quality and quantity of oocytes obtained. Selection of oocyte retrieval method aims at obtaining maximum number of good quality cumulus oocyte complexes (COCs) per ovary in short duration of time with minimum contamination. Oocytes have been retrieved from slaughter ovaries by aspiration of oocytes from follicles, slicing of ovary for oocyte collection, puncture of visible surface follicles and aspiration followed by slicing (Farahavar and Shahne, 2010). Even though slicing method yields more number of good quality oocytes, aspiration method is being widely used because of ease of procedure and speed of recovery (Hammad *et al*., 2014). The aim of this research is to determine the type of ovary (corpus luteum present or absent) that is most suitable to produce higher number of aspirable follicle and greater number and good quality cumulus oocyte complex for *in vitro* embryo production experiment.

2. MATERIALS AND METHODS

2. 1. Collection, processing and evaluation of ovaries

A total 40 numbers of Bovine ovaries were collected from 20 Boran heifers soon after the animals were slaughtered. In the laboratory, each ovary was trimmed to remove the surrounding

tissues and overlying bursa, then washed with normal saline containing antibiotic. The ovaries were then observed and categorized as corpus luteum absent (CL–) and corpus luteum present (CL+) groups (**Figure 1**) and the number of both types of ovaries were recorded. The follicles on the surface of the ovary for aspiration were counted and data were recorded.

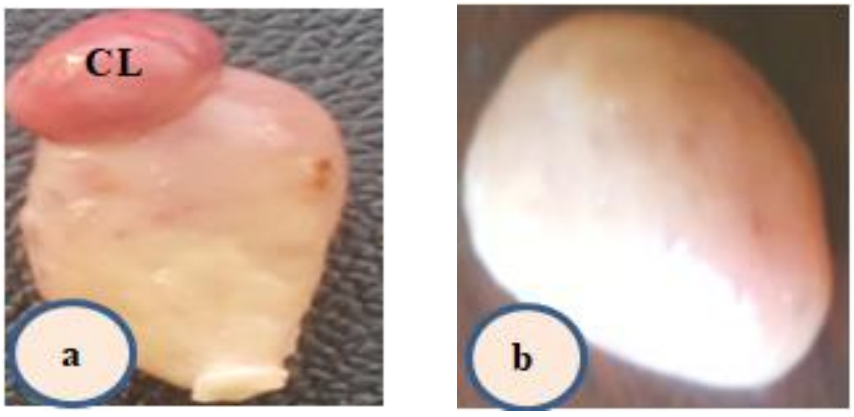

Figure 1. **(a)** Ovaries with corpus luteum and **(b)** ovaries without corpus luteum.

Figure 2. Representative photograph showing: **(a)** follicle aspiration **(b)** pouring in a four well Petri dishes and **(c)** microscopic evaluation of the COCs

Cumulus oocyte complexes (COCs) were collected by aspiration of surface follicles with a sterile 18 gauge needle attached to a 1 mL syringe containing the aspiration medium (**Figure 2**). All visible follicles on ovarian surface were aspirated. The contents of the syringe were poured in a four well Petri dishes and searched under a stereo zoom microscope for COCs at 40× magnification. The COCs were separated from the debris and picked individually on to another four well Petri dishes with washing medium.

2. 2. Qualitative and quantitative evaluation of aspirated follicle and collected oocyte

Cumulus oocyte complexes (COCs) were graded based on compactness, number of layers of cumulus cells and homogeneity of cytoplasm (Cetica *et al*., 1999). COCs with more than five layers of unexpanded cumulus and homogeneous ooplasm were considered as Grade I. Those with 3-5 layers of compact cumulus and evenly granular ooplasm were considered as Grade II, COCs with 1-2 layers of cumulus of partially denuded COCs with irregular ooplasm as Grade III and completely denuded oocyte were considered as Grade IV. They were graded according to Khandoker *et al*. (2001), as described in **Table 1**.

Table 1. Grading of COC quality (Khandoker *et al*., 2001)

Oocyte grade	Criteria
Grade I	oocyte surrounded with cumulous cells homogeneously (>3 layer of COCs)
Grade II	oocyte surrounded with cumulous cells partially (2-3 layers of COCs)
Grade III	oocyte not surrounded at all by cumulous cells
Grade IV	degeneration observed both in oocyte and cumulous cells

Quality grade I and II: normal (cultivable oocytes)
Quality grade III and IV: abnormal (non-cultivable oocytes)

Recovery rate

Oocyte recovery rate could be measured in terms of the oocyte recovery rate (ORR = number of oocytes per 100 follicles punctured), Oocyte yield from counted and aspirated follicle was calculated per ovary. Oocyte recovery rate (ORR) was determined as the following (Gabr and Gad, 2014):

$$ORR = \frac{Number\ of\ recovered\ oocytes}{Total\ number\ of\ follicles} \times 100\%$$

The oocyte quality index (overall quality) was calculated using the following formula **I = [(GI × 1 + GII × 2 + GIII × 3 + GIV × 4) / total number of oocytes recovered]** with

I = index and G = grade, as described by Duygu *et al*., (2013) and Kouamo *et al*., (2015). Index values that trend to one reflected good quality oocytes.

2. 3. Statistical analysis

The data collected were compiled, tabulated and analyzed in accordance with the objectives descriptive statistics and performed with the help of SPSS20.

3. RESULT

3. 1. Quantitative and qualitative evaluation of Boran heifer's slaughterhouse ovaries

The overall aspirated follicles, collected COCs and microscopic state (quality) of the retrieved COCs in both types of ovaries are given in **Table 2**. In this study, total aspirable follicles observed in both categories of ovaries were 583 from 40 ovaries and among them 381 was found to be in 25 CL– group ovaries and 202 in 15 CL+ group ovaries.

Table 2. Total number of follicles visualized, aspirated, and oocytes recovered by aspiration method from Boran heifers

Ovarian type	N	Total number of aspirated follicles	Total number of collected oocyte	COCs quality grade				OQI
				I	II	III	IV	
CL+	15	202	122	35	35	33	19	2.29
CL–	25	381	235	93	72	41	29	2.02
Total	**40**	**583**	**357**	**128**	**107**	**65**	**32**	**2.06**

Table 3. Mean (±SD) number of aspirated follicles and collected oocyte with different quality state oocyte from ovary bearing CL and without CL.

Type of ovary	Mean(±SD) follicle	Mean(±SD) oocyte	Oocyte quality grade (±SD)			
			I	**II**	**III**	**IV**
CL–	15.24±5.27	9.40±3.50	3.72±1.40	2.88±1.39	1.64±1.28	1.16±1.02
CL+	13.07±3.26	8.13±2.80	2.33±1.04	2.33±1.04	2.20±1.14	1.27±0.96

a: COCs with quality Grade I, b: COCs with quality Grade II, c: COCs with quality Grade III, and d: COCs with quality Grade IV

Mean number of aspirated follicles, collected COCs and quality of collected COCs in both types of ovaries are given in **Table 3**. In this study, an average of 15.24±5.27 aspirable follicles per ovary was recorded in CL− group and 13.07±3.26 aspirable follicles per ovary were recorded in CL+ group. Similarly, a little higher number of total COCs was collected from CL− group (9.40±3.50) than that of CL+ group (8.13±2.80).

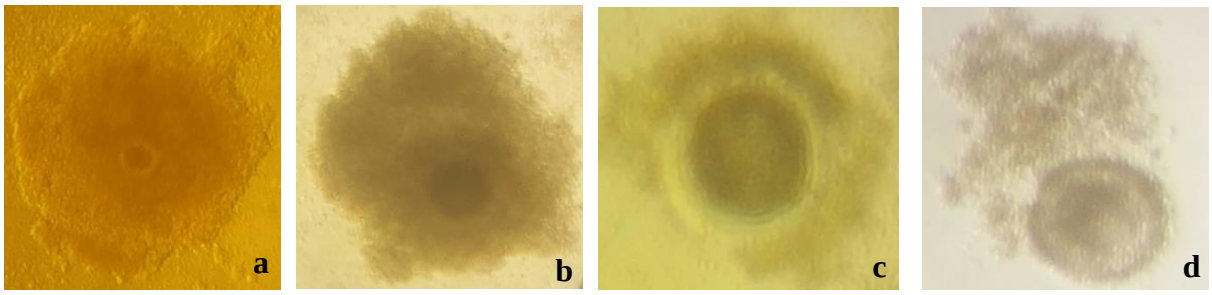

Figure 3. Grading of collected COCs, **(a)** Grade I, **(b)** Grade II, **(c)** Grade III, and **(d)** Grade IV

4. DISCUSSION

From the observation of this study, it was shown that among the 40 ovaries collected from slaughtered Boran heifers, 25 ovaries were obtained as without corpus luteum (62.5%) and 15 ovaries were obtained as with corpus luteum (37.5%). The numbers of ovaries having no corpus luteum usually were obtained from non-cyclic slaughtered heifers. Commonly, less reproductive performing cows were slaughtered and caused high possibility to get more CL− ovaries from the slaughterhouse during sample collection. The same trend of result was also reported in buffaloes by Khandoker *et al*., (2011) where among 136 ovaries, 93 were found without CL and the remaining 43 ovaries with CL. Similar results were also reported in goat (Asad, 2015; Saha *et al*., 2014; Mondal *et al*., 2008, and Islam *et al*., (2007).

The results obtained from this study shows that ovaries having no corpus luteum contributed larger number of aspirable follicles (15.24±5.27) than that of the ovaries with corpus luteum (13.07±3.26). The higher number of follicles found in without CL ovaries than those of with CL ovaries fits with the endocrinological explanation. The presence of corpus luteum in cyclic ovary causes a higher level of progesterone hormone production in which, giving a negative response to anterior pituitary gland for the restriction of gonadotrophin secretion, it leads to follicular degeneration and inhibition of the development of large follicles (Webb *et al*., 1999).

In noncyclic female, the absence of corpus luteum causes no negative effect of progesterone on anterior pituitary and thus estrogen-progesterone levels remain balanced which allow the growth of follicles. Ginther *et al*., (1996) stated that in ovaries without CL, the decrease in progesterone leads to increase in GnRH which stimulates the release of follicle stimulating hormone (FSH) and this hormone causes the rapid growth of ovarian follicles.

The results strongly supported by previous finding of the Mamy *et al*. (2016) on the effect of ovarian type and collection technique on the number of follicles and the quality of cumulus cell on cow, reports that higher number of follicles was aspirated and COCs collected per ovary

in without CL group than those of the with CL group. Asad (2015), also reported that, a higher number of follicles was aspirated per ovary in without CL group (2.92±0.08) than those of the with CL group (2.52±0.11) in goat. Similar findings also was noticed in buffalo ovaries by Khandoker *et al.* (2011) where a significantly higher number of follicles were collected in ovaries without CL (6.78±0.18) than in CL containing ovaries (4.09±0.26).

From this study, it was also found that more normal, quality Grade I (3.72±1.40) and quality Grade II (2.88±1.39) COCs were aspirated from ovaries without corpus luteum compared to quality Grade III (1.64±1.28) and quality Grade IV (1.16±1.02) COCs from ovaries with corpus luteum (**Table 3**). Nandi *et al.* (2000) stated that, when ovaries had a corpus luteum, the oocyte recovery rate decreased. This is because there will be restriction of follicular development as lutein cells occupy most of the ovary (Kumar *et al*., 2004). Hafez (1993) mentioned that in the presence of CL in ovary, the growth of follicle is inhibited while atresia is increased. These statements can be the physiological explanation for lower number of COCs in the with CL ovaries compared to without CL ovaries. Our findings were strongly supported by other researchers, they have done their research in goat (Asad, 2015; Khandoker *et al*., 2011; Mondal *et al*., 2008, and Islam *et al*., 2007), and in cow by Mamy *et al*. (2016).

Similarly, the higher number of normal COCs in CL absent group ovaries may be due to the hormonal effect of progesterone secreted by CL. When CL is absent in the ovary, progesterone which has role in follicular degeneration through negative effect could not be produced (Hafez, 1993). Thus, folliculogenesis can occur successfully and further there is more chance to produce high quality of COCs. Therefore, the types of ovary at the time of COCs collection have affected the quantity and quality of COCs recovered as well as usable oocytes in animals for use in IVEP program.

5. CONCLUSION

From this study results it can be concluded that comparatively higher number of ovaries were found without corpus luteum compared to ovaries with corpus luteum showing that the culled heifers were noncyclic due to various reasons and is important from economic point of view. Ovaries without CL contributed larger number of aspirable follicles per ovary compared to ovaries with CL. Furthermore, comparatively higher number of total COCs and superior quality of COCs (A and B grade) were possible to obtain from without CL ovaries, suggested to be suitable for collecting COCs for initiating and optimizing *in vitro* embryo production experiment alike in Ethiopia, having huge cattle population and animal slaughtering that is more common for various reasons, like to export or for domestic consumption.

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