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Effects of ovarian phase on oocyte quality in cows

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Abstract

The effect of ovarian phase on oocyte quality in slaughterhouse material of cows was studied. Cumulus-oocyte complexes (COCs) were aspirated from all visible follicles. COCs were graded on the number and intensity of the cumulus cells and the homogeneity of the ooplasm as A (4 – 5 layers compact cumulus with a homogeneous ooplasm), B (1 - 2 layers compact cumulus with homogeneous ooplasm having a coarse appearance), C (slightly expanded cumulus with irregular cluster ooplasm), and D (denuded oocyte or expanded cumulus, irregular ooplasm). The overall aspiration rate of COCs was 37.2%. The aspiration rate was significantly higher (P<005) in follicular (41.2%) than in luteal phase (28.6%). The percentage of grade A and B oocytes was significantly (P<005) higher in the follicular phase (20.4 and 28.6%) than luteal phase (11.1 and 22.2%). The proportion of grade A COCs in the indigenous cows was significantly (P<005) higher (29.0%) than in crossbred cows (8.3%). Ovaries with diameter of 2.0 cm or more had higher proportion (P<005) of grade A and B COCs than others. It is suggested that follicular phase of the sexual cycle had significant effect on COCs quality. (*Bang. vet.* 2023. Vol. 40, No. 1 – 2, 1 – 7)

Introduction

Embryo transfer from genetically upgraded animals to the indigenous cows is used for genetic improvement. The embryo production rate depends on the quality of oocytes, which is important for *in vivo* or *in vitro* maturation of oocyte and embryo production. Despite advances, the proportion of transferable embryos derived from *in vitro* fertilization (IVF), is still low (Ferré *et al.* 2020). It is more related to the ovarian phase and source of oocytes than to the conditions of *in vitro* fertilization and culture (Bols *et al.* 1996) or the method of *in vitro* maturation of oocytes (Leibfried-Rutledge *et al.* 1987). According to Reis *et al.* (2002), Simmental heifers have more collected oocytes when the corpus luteum (CL) is active. Similar findings in Holstein cows were reported by Reis *et al.* (2006) and by Manjunatha *et al.* (2007). Conversely, de Wit *et al.* (2000) showed no difference in the quality and number of oocytes collected from abattoir ovaries in the follicular, early luteal, and late luteal phases. Determination of the quality of oocytes is a priority for *in vitro* fertilization. Oocyte quality may depend on their age (Yamamoto *et al.*, 2010), the stage of the oestrous cycle (Wurth *et al.*, 1994),

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hormonal patterns (Kruip and Dieleman, 1982), biochemical characteristics of the follicular fluid (Wise, 1987), diameter of the follicles (Wurth *et al.*, 1994), atresia grade of the follicle (Wurth and Kruip, 1992), and ovarian morphology (Gandolfi *et al.*, 1997). Oocyte quality may coincide with its developmental competence. Several strategies were employed to predict the *in vitro* embryo production potential of oocytes. Most are based on morphological criteria, but the relationship between morphological and functional criteria does not always provide results as expected. Besides the predictive value, the analysis of oocyte quality may provide useful information on the mechanisms underlying oocyte maturation and developmental competence and suggest new possibilities to improve the efficiency of embryo production. A detailed comparison of morphological features between *in vivo* and *in vitro* maturation of cattle oocyte has been reported by de Loos *et al.* (1992). There is no comprehensive report on oocyte quality for IVF in Bangladesh. The aim of the study was to determine the quality of cattle oocytes from the slaughterhouse-derived ovaries considering the age, breeds, ovarian and follicular sizes.

Materials and Methods

Preparation of physiological saline

One litre of physiological saline was prepared by dissolving 9.0 gm of sodium chloride in 1000 ml distilled water and stored at room temperature.

Preparation of phosphate buffer saline (PBS)

The ingredients of PBS are shown in Table 1. One ml Pronapen vet-40 lac (4 lac dilution) was added and stirred. Aliquot of sterilized solutions was kept in sterilized bottles for further use.

Table 1: Ingredients and amount of PBS

Ingredients	Amounts
NaCl	10 gm
KCL	0.25 gm
Na_2HPO_4	1.44 gm
KH_2PO_4	0.25 gm
Distilled water	1 litre

Collection and transport of ovary

Ovaries were obtained from the slaughterhouses and kept at room temperature in physiological saline (0.9% NaCl) for up to four hours until transfer to the laboratory. Breed, age and body weight of cows, and ovarian phase and presence of CL were recorded. After slaughtering, left and right ovaries were collected, and kept in a small poly bag containing physiological saline in an icebox. In the laboratory ovaries were washed three times with physiological saline and twice with PBS. The length and

width of each ovary and number of small, medium and matured follicles was counted and recorded (Fig. 1). Oocytes were collected from visible follicles using an 18-gauge needle with 10 mL syringe. About 0.5 ml of PBS solution was taken into the syringe, and follicular fluid was aspirated and transferred to a conical flask. Follicular fluid was put in a searching Petri dish and oocytes were collected by a micropipette and transferred to another Petri dish and graded for quality, counted under the stereo microscope (Novel Biological Binocular Microscope XSZ-107T China).

Grading of oocytes

Oocytes were graded morphologically based on the number and intensity of the cumulus cells and the homogeneity of the ooplasm as described by de Loos *et al.* (1989) (Fig. 2) and below.

Grade-A: Highest quality oocyte. Compact cumulus (4 – 5 layers) with a homogeneous ooplasm.

Grade-B: Compact cumulus of one to two layers with homogeneous ooplasm having a coarse appearance.

Grade-C: Less compact (slightly expanded) cumulus with irregular ooplasm containing dark clusters.

Grade- D: Denuded oocyte or expanded cumulus, irregular ooplasm.

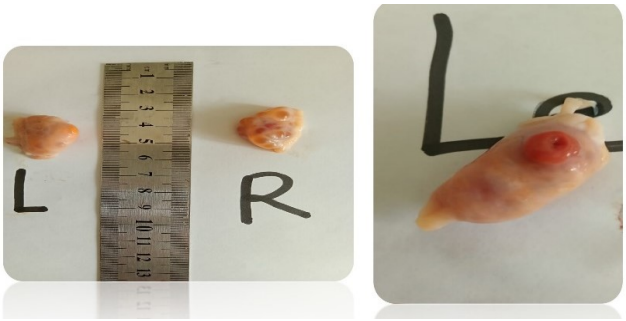

Fig. 1: Measurement of ovary and counting follicles

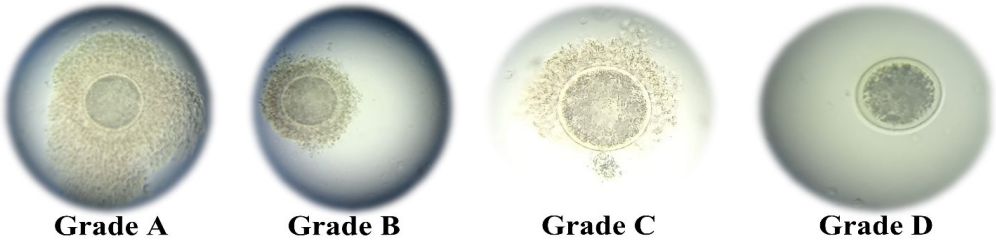

Fig. 2: Grading of COCs

Statistical analysis

The data was coded in a Microsoft Excel sheet. The rate was expressed as a percentage (%). The analysis of variance was done to test the significance by SPSS software (Anon, 1996). A statistical significance was considered at P<0.05.

Results and Discussion

Effects of ovarian phase on oocyte quality

The aspiration rate and the oocyte quality are shown in Table 2. The aspiration rate was significantly higher (P<005) in follicular phase (41.2%) than in luteal (28.6%). The overall aspiration rate was 37.2%. Khondokar *et al.* (2016) reported an aspiration rate 48.6%. These findings agree with Asad (2015), who reported more follicles aspirated in the ovary without CL in goats. Khandoker *et al.* (2011) reported significantly more follicles collected in ovaries without CL (6.8 ± 0.2) than with CL (4.1 ± 0.3). Nandi *et al.* (2000) stated that when CL is present, the oocyte recovery rate is decreased because follicular development is restricted as lutein cells occupy most of the ovary (Kumar *et al.,* 2004). Hafez (1993) mentioned that in the presence of CL, the growth of follicles is inhibited. The percentage of grade A and B oocytes was significantly (P<005) higher (20.4 and 28.6% *vs* 11.1 and 22.2%) in the follicular than the luteal phase, whereas grade C and D were significantly higher (27.8 and 39.9% *vs* 22.4 and 28.6%) in the luteal phase. Boni *et al.* (2012) found that the proportion of grades A, B, and D were 28.7, 23.1, and 3.4%, respectively. Wurth *et al.* (1994) stated that the ovarian phases influence follicular quality and quantity. Webb *et al.* (1999) reported that progesterone from the CL inhibits the anterior pituitary gland, restricts gonadotrophin secretion and inhibits the development of large follicles. In noncyclic females, the absence of the CL means that oestrogen-progesterone concentrations remain balanced, which allows the growth of follicles. Ginther *et al.* (1996) stated that in ovaries without CL, the lower progesterone leads to an increase in GnRH, which stimulates the release of follicle-stimulating hormone (FSH) that causes the growth of ovarian follicles. Chohan and Hunter (2003) found the highest developmental competence in oocytes collected from metoestrus cows while the lowest was in oocytes from pregnant and anoestrus cows.

Table 2: Quality of oocytes in relation to ovarian phases

Ovarian phase	No. of Follicles Counted			Total	Aspiration Rate % (n)	Grading of oocytes % (n)			
	Small (<3mm)	Medium (3-6mm)	Large (>6mm)			Grade A	Grade B	Grade C	Grade D
Follicular (n = 16)	70	34	15	119	41.2 (49)[a]	20.4 (10)[a]	28.6 (14)[a]	22.4 (11)[a]	28.6 (14)[a]
Luteal (n = 10)	44	15	4	63	28.6 (18)[b]	11.1 (2)[b]	22.2 (4)[b]	27.8 (5)[b]	39.9 (7)[b]
Overall (n = 26)	114	49	19	182	37.2 (67)	17.9 (12)	26.9 (18)	23.9 (16)	31.3 (21)

In a column, figures with different superscript differ significantly (P<0.05)

The effect of different factors on COCs quality

The rate of COCs quality in relation to the age, breed and ovarian size is shown in Table 3. The age of animals was categorized as 1.5 to 2 years and more than 2 years old. The proportion of grade A and B COCs was not significantly different. Cows were categorized as local indigenous, non-descriptive, and crossbred. The proportion of grade A and B COCs in the indigenous cows was significantly (P<005) higher than in crossbred cows. Ovaries 2cm or more had significantly (P<005) higher proportion of grade A and B COCs. Follicle development can be affected by season, heat stress, post-partum interval, hormonal status, milk yield, energy balance, nutrition, oestrous cycle or genetics. The effect of the stage of the oestrous cycle on oocyte quality and on *in vitro* embryo production efficiency was evaluated by Wurth *et al.* (1994). Gandolfi *et al.* (1997) proposed an ovarian evaluation criterion on the basis of the number and size of the visible follicles. Ovaries were separated into three categories: (A) a follicle >10 mm in diameter, (B) more than 10 follicles 2 to 5 mm in diameter and no follicles >10 mm, and (C) fewer than 10 follicles 2 to 5 mm in diameter and no follicles >10 mm. Fewest COCs were collected from the category C. In addition, the COCs of this category showed the lowest *in vitro* embryo production efficiency and yielded blastocysts with the fewest cells. There is no solid explanation for this finding, but diet, environment, age, season, or individual variations of growth factors, which mediate intra-ovarian stimuli, have been suggested as possible reasons.

Table 3: COC quality in relation to different parameters

Parameter	Variable	Number of oocytes aspirated	Quality of oocytes % (n)			
			Grade A	Grade B	Grade C	Grade D
Age (years)	1.5-2.0	43	18.6 (8)	25.6 (11)	20.9 (9)[a]	34.9 (15)[a]
	>2.0	24	16.7 (4)	29.2 (7)	29.2 (7)[b]	25.0 (6)[b]
Breeds	Indigenous	31	29.0 (9)[a]	16.1 (5)[a]	19.4 (6)	35.5 (11)
	Cross	36	8.3 (3)[b]	36.1 (13)[b]	27.8 (10)	27.8 (10)
Ovary size (cm)	1.0-2.0	22	9.1 (2)[a]	13.6 (3)[a]	40.9 (9)[a]	36.4 (8)
	2.1-<2.5	20	15.0 (3)[a]	25.0 (5)[b]	30.0 (6)[b]	30.0 (6)
	≥2.5	25	28.0 (7)[b]	40.0 (10)[c]	4.0 (1)[c]	28.0 (7)

In a column, figures with different superscript differ significantly (P<0.05)

Conclusions

The ovarian phase has a significant effect on COC quality. Age of cows has no effect on COC quality, but ovary size affects the quality of COCs. Further study is required for *in vitro* quality embryo production using collected COCs on slaughterhouse materials.

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