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Effect of Season of the year and Ovarian Structures on Oocytes Recovery Rate, Quality and Meiotic Competence in Egyptian Buffaloes

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Abstract: The present study aimed to evaluate the effect of season of the year and ovarian structure (corpus luteum) on the recovery rate, quality and maturation of Egyptian buffalo oocytes. For this purpose, ovaries were collected from slaughtered buffaloes and oocytes were scored after recovery by aspiration. Oocytes were matured *in vitro* for 24 h and evaluated for nuclear maturation after staining by 1% orcein stain. Results showed a significantly ($P<0.05$) higher recovery rate, quality and maturation rate of buffalo oocytes from ovaries collected during Spring season (high ovarian activity) than those in Summer season (Low ovarian activity). Presence of luteal structure had no significant effect on oocyte yield and quality, though a significantly ($P<0.05$) higher maturation rate was recorded in the absence of corpus luteum on the ovary. In conclusion, it was determined that season and luteal tissue at the time of collection influences the recovery, quality and meiotic maturation of Egyptian buffalo oocytes and their liability for use in IVF programs.

Key words: Buffalo · Corpus Luteum · IVM · Oocytes · Season

INTRODUCTION

Recovery of a large number of oocytes from ovaries of slaughtered or live animal (s) as well as its quality is a prerequisite for *in vitro* maturation (IVM) and fertilization (IVF) and successful production of IVM-IVF embryos. Buffalo ovaries contain thousands of oocytes, but only a small number is utilized during their reproductive lifespan and most of these are wasted after slaughtering. Additionally, there are many factors including age, ovarian size, presence or absence of corpus luteum (CL) and season influence the quantity and quality of recovered oocytes per ovary from slaughtered animals [1, 2].

Although, buffaloes are polyestrous, they exhibit a distinct seasonal variations in breeding activity [3, 4]. A lower yield of oocyte per ovary was recorded in buffaloes during hot season [2] in association with relatively inactive ovarian status [5] and this might be an attributable factor for the seasonality of breeding in this species.

To the best of the authors' knowledge, there is no detailed study regarding the effect of reproductive status on the oocytes recovery rate, morphology and appearance in buffalo. Moreover, observations in bovines are contradictory [6] and needs further investigations. While some records found that the stage of estrous cycle has no effect on bovine oocytes quality or its developmental potentials [7], others isolated a higher proportion of good quality oocytes with higher rate of developmental competence at the end of the luteal phase in cows [8]. Several researchers recorded that CL bearing ovary contains more follicles [9] and influence the recovery rate of oocytes, IVM and IVF [10]. Fewer medium and large follicles were collected from pregnant than cyclic cows [11].

Therefore, the present study was designated to investigate the effect of season and presence CL on oocyte yields, quality and consequently their maturation rate in Egyptian buffaloes.

MATERIALS AND METHODS

Experimental Designs

Experiment I: Effect of the season on recovery rate, quality and maturation rate of oocytes in Egyptian buffaloes.

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Ovaries were obtained from slaughtered buffalos during spring (n=123) and summer (n=113) seasons of the year to assess the effect of the season on recovery rate, quality and maturation rate of collected oocytes.

Experiment II: Evaluation the relationship between the existence of corpus luteum and oocytes yield and maturation *in vitro*.

Ovaries were obtained from slaughtered buffalos and categorized according to the presence (n=126) or absence (n=106) of corpus luteum. Oocytes were aspirated from ovaries and evaluated for the effect of corpus luteum existence on the recovery rate, quality and maturation rate of collected oocytes.

Collection of Ovaries: Ovaries were collected immediately after slaughtering of buffaloes at local abattoir (El-Moneeb, Giza) and were transported to the laboratory (National Research Centre, Dokki, Giza) within 1-2 hours in a thermos flask containing phosphate buffer saline (PBS, pH 7.2) supplemented with antibiotics (100.000 IU penicillin and 100 mg streptomycin/l) at 30-38°C. Ovaries dissected free of adipose and connective tissues were then washed several times in warm PBS (30-38°C).

Collection and Classification of Oocytes: The oocytes were harvested from all visible ovarian follicles by aspiration using 18-gauge needle fixed on 10 ml disposable syringe filled with 1 ml of the aspiration medium (0.01M PBS at pH 7.2, supplemented with 3% heat inactivated fetal calf serum (FCS) or 3 mg/ml bovine serum albumin (fraction V) and 50 µg/ml gentamycin). Oocytes were recovered under stereo microscope (10×) by sterile Pasteur pipette, washed 2-3 times in TCM 199 (Earle's salt) enriched with 10% FCS, evaluated morphologically according to the criteria of cumulus investment and classified into four classes (A; completely invested with cumulus cell layers, B; partially invested with cumulus cells, C; denuded oocytes and D; degenerated oocytes).

***In vitro* Maturation of Oocytes:** The recovered oocytes (Class A and B) were matured *in vitro* using TCM 199 supplemented with 10% FCS, 50 µM cysteamine and 50 µg/ml gentamycin for 24 hours (39°C, 5% CO_2 in air with 95% relative humidity).

Chromosome Preparation: At the end of the culture period, oocytes were subjected to chromosomal preparation according to the procedure described by Tarkowski [12]. Briefly, cumulus cells were removed mechanically by gentle pipetting, oocyte was transferred to 1% hypotonic sodium citrate solution for 10 min. and placed on a microscope slide with a minimal amount of hypotonic solution. Three drops of methanol/glacial acetic acid (3:1) fixative were dropped onto the oocytes before staining with 1% orcein stain. The evaluation of nuclear maturation was performed according to the method described by Hamam *et al.* [13].

Statistical Analysis: Data were presented as mean (±S.E.), tabulated and analyzed for impact of season (spring vs. summer) and corpus luteum (presence vs. absence) on examined parameters by Student *t*-test using SPSS (ver.14) statistical software. Differences were considered to be significant at $P<0.05$.

RESULTS

Experiment I: Effect of the season on recovery rate, quality and maturation rate of oocytes in Egyptian buffaloes.

The effect of season on the recovery and maturation rates of buffalo oocytes is presented in Tables1 and 2. The mean oocyte recovery rate per ovary was nearly similar during spring (high ovarian activity) and summer (low ovarian activity) seasons (2.57±0.23 vs. 2.60±0.21). However, ovarian samples collected during summer months were characterized by a significant ($P<0.05$) higher rate of degenerated oocytes (class D), lower number of good quality oocytes and lower the maturation rate than those collected during spring months (19.43±3.12 vs. 10.28±1.94, 72.77±2.28 vs. 81.69±2.95 and 63.40 ±2.15 vs. 71.49 ± 1.20, respectively).

Experiment II: *In vitro* evaluation the relationship between the existence of corpus luteum and oocytes yield and maturation.

Data regarding the effect of presence or absence CL on oocyte recovery and Maturation rates is shown in Tables 3 and 4. Statistical analysis did not reveal significant differences between ovaries with and without CL in terms of the yield and quality of oocytes in samples collected under the conditions of the present investigation. On the other hand, oocytes collected from ovaries without CL were associated with a significant ($P<0.05$) higher maturation rate rather than those had CL (77.77± 2.12 vs. 64.03± 2.07).

Table 1: Effect of breeding season on the number and quality of recovered buffalo oocytes

Season	No. of ovaries	No. of oocytes	Number of recovered oocytes per ovary	COC NO. (% ± S. E)	PCO No. (% ± S. E)	Denuded No. (% ± S. E)	Degenerated No. (% ± S. E)	Good quality No. (% ± S. E)
Spring	123	306	2.57±0.23	137 (44.67±4.32)	114 (37.02±4.55)	24 (8.26±2.56)	31 (10.28±1.94)	251 (81.69±2.95)*
Summer	113	307	2.60±0.21	105 (32.53±4.77)	120 (40.25±2.99)	24 (7.80±0.93)	58 (19.43±3.12)*	225 (72.77±2.28)

Data presented as mean (±S.E).* indicated significant (P<0.05) difference between spring and summer seasons and analyzed by student *t*-test. COC: cumulus oocyte complex and PCO: partial cumulus oocyte.

Table 2: Effect of breeding season on the maturation rate of buffalo oocytes *in vitro*

		Stage of nuclear development						
Season	Total No. of oocytes	GV No. (%)	GVBD No. (%)	MI No. (%)	AI No. (%)	MII No. (%)	TI No. (%)	Maturation rate No. (%)
Spring	164	32 (19.51)	7 (4.26)	4 (2.13)	4 (2.44)	92 (56.09)	25 (15.24)	117 (71.49±1.20)*
Summer	143	32 (22.38)	10 (6.99)	5 (3.49)	6 (4.19)	74 (51.75)	16 (11.19)	90 (63.40±2.15)

Data presented as mean (±S.E).* indicated significant (P<0.05) difference between spring and summer seasons and analyzed by student *t*-test. GV: germinal vesicle stage; GVBD: germinal vesicle break down; MI: metaphase I; AI: anaphase I; MII: metaphase II; TI: telophase I stage

Table 3: Effect of the presence or absence of corpus luteum on the recovery and quality of buffalo follicular oocytes

Corpus luteum	No. of ovaries	No. of oocytes	Average No. of oocytes/ ovary	COC NO. (%)	PCO No. (%)	Denuded No. (%)	Degenerated No. (%)	Good quality No. (%)
CL. presence	126	298	(2.37±0.14)	94 (30.00±5.00)	124 (43.59±6.41)	32 (10.39±1.47)	48 (16.02±1.20)	218 (73.59±1.41)
CL. absence	106	244	(2.27±0.02)	98 (37.99±6.79)	70 (30.30±4.87)	26 (9.78±2.37)	50 (21.91±3.84)	168 (68.29±1.96)

COC: cumulus oocyte complex, PCO: partial cumulus oocyte)

Table 4: Effect of absence and presence of corpus luteum on maturation rate of buffalo oocytes *in vitro*

		Stage of nuclear development						
Corpus luteum	Total No. of oocytes	GV No. (%)	GVBD No. (%)	MI No. (%)	AI No. (%)	MII No. (%)	TI No. (%)	Maturation No. (%)
CL. presence	143	39 (27.27)	3 (2.09)	5 (3.49)	5 (3.49)	74 (51.75)	17 (11.89)	91 (64.03±2.07)
CL. absence	161	27 (16.77)	3 (1.86)	3 (1.86)	2 (1.24)	97 (60.25)	29 (18.01)	126 (77.77±2.12)*

Data presented as mean (±S.E).* indicated significant (P<0.05) difference between ovarian samples with or without corpus luteum analyzed by student *t*-test. GV: germinal vesicle stage; GVBD: Germinal vesicles break down; MI: metaphase I; AI: anaphase I; MII: metaphase II; TI: telophase I.

DISCUSSION

In the present study, oocytes quality and maturation rate was significantly higher during the season associated with peak ovarian activity (Spring) than those collected during the low breeding season (Summer). A similar trend was recorded in previous studies in cattle [13] and buffalo [8, 14, 15]. Fewer follicles on ovaries [16] and lower oocyte yield per ovary [15] were found on buffalo slaughtered during hot summer season beside it's unfavorably influence on quality of oocytes [14]. Buffaloes [17, 18] and cows [19] under heat stress produced fewer good quality oocytes than unstressed animals. Former study evidenced that heat stress can alter endocrine patterns and reduced follicular development [20] and alter phospholipids composition of oocytes [21]. Such effect might lead to depression in oocyte quality [22] and relatively inactive status of ovaries and probably responsible for the seasonality of breeding in buffalo species [5]. What is more, high ambient temperature and humidity had deleterious effects on oocyte capability for maturation and fertilization *in vitro* [22] and embryo viability of super-ovulated Holstein cows [23]. Shi *et al.* [24] referred the role of seasonal variation on *in vitro* embryo production to the differences in susceptibility of various male gametes to reflect seasonal divergences in their capability for fertilization and competence to develop to the blastocyst stage.

Very few studies have been dealt with follicular development in relation to the reproductive status of the ovary in buffalo and contradicting. In the current work, a higher percentage of maturation was obtained from ovaries in absence of CL. Similar results were recorded in buffalo by Jamil *et al.* [2]. Das *et al*. [25] and Raza *et al.* [26] recorded that the presence of a CL significantly reduces the number of ovarian follicles as well as the quality of the oocytes. This is because follicular development is restricted, as lutein cells occupy most of the ovary [27]. Corpus luteum may act on follicles to alter their growth rate and result in atresia [28]. However, the exact reason for the superiority of the ovary without a CL over those of CL-bearing ovary is not clear and need

further investigation. On the other hand, earlier studies affirmed higher maturation rate of oocytes recovered from ovaries having active structure, i.e. corpus luteum [29, 30] or dominant follicle [30] and its absence was associated with poor in vitro oocytes development in bovine [30]. Previous studies in buffalo [9] and cows [31] recorded that the presence of a CL stimulates follicular development and accordingly CL-bearing ovary contains more follicles. In conclusion, it was determined that season and ovarian status at the time of collection significantly affects the recovery, quality and meiotic maturation of buffalo oocytes for use in IVF programs.

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