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Effect of Season and Ovarian Morphology of Egyptian Buffalo on Oocyte Quality and In Vitro Maturation Rate

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ABSTRACT

The aim of the current study was to investigate the effect of season, follicle size and corpus luteum (CL) on yield, quality and *in vitro* maturation of Egyptian buffalo oocytes. Ovaries were collected from abattoirs all over the year and cumulus oocytes complexes (COCs) were aspirated from visible small (≤3 mm), medium (3-6 mm) and large (>6 mm) follicles of normal ovaries. Ovaries were classified according to bearing mature CL3 into with and without CL. Oocytes quality were classified into good, fair, poor and denuded. Only good and fair quality oocytes were used for in vitro maturation in TCM-199 medium supplemented with 10% FCS, 10 µg/ml ovine FSH, 10 IU/ml hCG, 1 µg/ml Estradiol and 1% antibiotic antimycotic mix. Results revealed that a significant difference between the four seasons with significant increase of oocyte yield, quality and maturation for winter and spring than autumn and summer seasons. Oocytes aspirated from medium sized follicles (3-6 mm) were of higher in quality and maturation rate than large (> 6 mm) and small size follicles (≤ 3 mm). Recovery rate, quality and maturation rate of oocytes were higher for ovaries without CL than ovaries with CL. In conclusion, the winter season, medium sized follicles and ovaries without CL showed positive effects on recovery rate, quality and *in vitro* maturation of buffalo oocytes.

Key words: *Oocytes, season, follicle size, in vitro maturation, buffaloes*

INTRODUCTION

Lower reproductive pattern in buffalo related to high percentage of atretic follicles, little number of follicles, weak superovulation response, seasonal breeding, silent heat, delayed onset of maturity and long calving interval (Haldar and Prakash, 2007; Nandi *et al.*, 2002). Last years, efforts have been done for increasing the reproductive pattern of these species using biotechnology (Madan *et al.*, 1994). Assisted reproductive technologies (ART) such as in vitro fertilization (IVF) have been introduced for increasing reproductive efficiency and produce embryos of high genetic quality used for embryo transfer (Nandi *et al.*, 2002). The oocyte quality can play an essential role in oocyte developmental competence *in vitro* (Amer *et al.*, 2008). Buffalo ovaries yielding low number of oocytes used for the application of IVF procedures are more questionable (Chohan and Hunter, 2003). Low oocyte yielding has been attributed to a low number of primordial follicles (10,000 to 19,000) in buffalo ovary compared to cattle (150,000) (Danell, 1987). The *in vitro* embryo production and blastocyst development are still very poor (10-30 %) and need a lot of challenges (Raghu *et al.*, 2002). The buffalo is a polyestrous animal but tend to be seasonal breeder, the yielding and quality of oocytes decrease during summer season (Nandi *et al.*, 2001).

The reproductive efficiency of buffalo was affected by environmental factors such as temperature, relative humidity, day length and photoperiod either alone or in combination (Ribeiro *et al.* 2003). There was a strong relation between poor nutrition in summer and

high incidence of ovarian inactivity which is the main cause of infertility in buffaloes in Middle Egypt (Ali *et al.*, 2009).The ovaries collected from abattoirs were having different structures of the estrus cycle, including follicles at different diameters and corpora lutea (Hosseini et al ., 2008). The oocytes can be aspirated from follicles (2-6) mm for in vitro maturation (Raghu *et al.*, 2002). The oocytes can be affected by the follicular development and formation of mRNA which accumulates in the oocytes for early development of blastocyst (Aksu *et al.*, 2015).

The success of *in vitro* maturation (IVM) of buffalo oocytes need to adjust maturation medium, protein and hormones supplements and quality of cumulus-oocyte complexes (COCs) which will improve subsequent fertilization and *in vitro* culture (Bavister and Rose-Hellekant, 1992). The oocyte quality can be assessed by their ability to mature, fertilize and produce blastocyst (Hussein *et al.*, 2006). Recently, the quality of oocytes and *in vitro* embryo production (IVEP) can be increased in river buffaloes by superstimulation of follicular activity by FSH followed by GnRH (Sakaguchi *et al.*, 2019) or addition of Retinoic acid (vitamin A metabolite) to the media increased gene expression, maturation and decrease the ROS (Gad *et al.*, 2018). The ovarian structures, morphology and CL are still requiring more investigation and study for improvement of oocyte quality and in vitro maturation in buffalo (Singh and Adams, 2000). Therefore, the present study aimed to investigate the maturation rate of oocytes in Egyptian buffaloes in different seasons of year and examine the impact of follicle size and presence/absence of mature corpus luteum (CL3) on oocytes yield and quality.

2. MATERIALS AND METHODS

This study was carried out at the Dept. of Theriogenology, Faculty of Veterinary Medicine, University of Sadat City, Egypt during the period from September 2017 to November 2018.

2.1. Collection of ovaries

Ovaries from apparently normal reproductive organs of adult Egyptian buffaloes of unknown reproductive history slaughtered in Elbatanoon and Shibin Elkom abattoirs belonged to Menoufia province were collected within 10 min after slaughtering according to Lee and Fukui, (1995) and transported to laboratory in a thermos flask containing warm saline solution (0.9%) supplemented with antibiotics (100.000 IU penicillin and 100 mg streptomycin/L) at 25-30°C within 1-2 h. Ovaries were dissected from adjacent tissue and washed three times in sterile physiological saline to remove any contamination on the ovarian surface and then kept in saline until cumulus-oocytes complexes (COCs) were recovered by aspiration technique.

2.2. Oocytes collection and classification

Cumulus-oocytes complexes (COCs) were aspirated from visible follicles (2-8 mm) using an 18- gauge needle attached to a 10-ml disposable plastic syringe primed with 1 ml of pre-warmed modified phosphate buffered saline (P-3813, Sigma) supplemented with 2mg/ml BSA (A-9647, Sigma).The recovered (COCs) were examined under stereo microscope (10×) (Optitech, SZ-217, 60X, Germany) and graded according to their quality on the basis of cumulus investment and granularity of ooplasm according to Leibfried and First, (1979) into four grades: (Grade I good, homogenous granular ooplasm and surrounded by compact dense 3-6 cumulus cell layers), (Grade II Fair, having 1-3 cumulus cell layers), (Grade III Poor, Partially denuded oocytes), (Grade IV Bad, denuded oocytes with degenerated un even ooplasm). Oocytes of the first two categories were selected for further experiments.

2.3. In vitro maturation of oocytes

The good quality selected buffalo oocytes randomly assigned to be cultured in drops of 100 µl maturation medium using TCM 199 with Earle's salts (M-4530,Sigma) supplemented with 10% FCS (F-7524,Sigma) , 10 µg/ml ovine FSH (F-8174), 10 IU/ml hCG (Chorimon, IBSA, Switzerland), 1 µg/ml Estradiol (E-8875) and 1% antibiotic antimycotic mix (A-5955, Sigma), covered with sterilized mineral oil (M- 8410, Sigma) in culture dishes (35mm, Nunc, Roskilde, Denmark) for 24 h at 38.5 ◦C under 5% CO2 in air with maximum humidity (>95%).

Experiment 1: Effect of the season on recovery rate, quality and maturation of oocytes. The ovaries were collected over four seasons (summer, autumn, winter, spring).

Experiment 2: Effect of follicles size on recovery rate, quality and maturation of oocytes. Cumulus oocytes complexes (COCs) were aspirated from small (≤ 3 mm), medium (3-6 mm) and large (>6 mm) follicles according to **Raghu *et al.*, (2002).** The follicle size was measured by digital Vernier Caliper.

Experiment 3: The effect of the presence of CL on the ovary on the oocyte yield, quality and maturation. The collected ovaries were grouped based on the presence or absence of corpus luteum (CL) into 2 types: Type I (ovaries with corpus luteum), Type II (ovaries without corpus luteum). The numbers of follicles per ovary for each ovarian type were recorded. (1.8 follicle per ovary with CL and 2.4 follicle per ovary without CL).

2.4. Statistical analysis

Statistical analysis was performed using (GraphPad prism 5 software Inc., La Jolla, CA). Statistical significance between groups was detected using Student's t-test (for two groups) or one-way ANOVA followed by Bonferroni's multiple comparison test (for more than two groups. Data are presented as means± standard errors of means (SEM). Values of $p < 0.05$ were considered significant.

3. RESULTS

Effect of the season on the quality of oocytes and their maturation rate

The effect of season on the yield, quality of oocytes and their maturation rate are presented in Table (1). The average number of recovered oocytes per ovary fluctuated during different seasons with the least value during summer (0.88) and the highest value during autumn (1.59) followed by winter (1.42). For grade I oocytes, the frequency recorded was markedly higher (P<0.05) during winter and spring (8.3±1.29 and 9.41±1.32) respectively. The frequency of grade II oocytes was higher (P<0.05) during spring (10.5±2.08). Grade III and IV were fairly higher (P< 0.05) in summer and autumn. The selected oocyte maturation differs significantly between seasons and the results showed that the winter and spring seasons characterized by the highest maturation rate (59.67 %) and (48.54%) respectively, then autumn (44.26%) and summer (28.82%) (Figure 1).

Effect of the follicle size on the quality of oocytes and their maturation rate

Results showing the influence of follicle size on the quality of oocytes and maturation rate of buffalo oocytes are presented in Table (2). The oocytes harvested from ovaries containing follicular diameter ranged from (3-6mm) were of good quality (grade I, II) (P<0.05) than small (≤3) mm and large sized (>6) mm diameters. Grade III, IV were lower (P<0.05) from ovaries containing large sized follicles (>6) mm in diameter. There was a significant increase (P<0.05) in the maturation rate of oocytes collected from ovaries carrying medium sized follicles (3-6mm) (66.8%) compared to small and large sized follicles.

The effect of the CL on the ovarian surface on the oocyte yield and quality

Oocyte yield and quality as affected by presence or absence of CL on the ovaries were presented in Table (3). The ovaries without CL recorded a higher value for oocyte yield (2.1) compared to the ovaries with CL (1.3). The quality of the recovered oocytes (I, II, III) showed a significant (P<0.05) higher values for ovaries without CL than those with CL. There was a significance difference (P<0.05) in maturation rate of oocytes recovered from ovaries without CL (55.6%) than ovaries with CL (42%).

Table (1): The relationship between the seasons, yielding, quality of oocytes and their maturation rate in Egyptian buffalo (mean ±SEM).

Season	No. of ovaries	oocyte number (total) Mean ±SE	No. of oocytes / ovary	Oocyte quality				Maturation rate
				Grade I Good No (%) mean±SEM	Grade II Fair No (%) mean±SEM	Grade III Poor No (%) mean±SEM	Grade IV Denuded No (%) mean±SEM	
Summer	236	208 20.7±2.4	0.88	50 (24.15 %) 5±0.68^{a}	61 (29.47%) 6.1±1.1^{a}	48 (23.19%) 4.8±0.84	49 (23.19%) 4.8±0.55	28.82^{a} 3.2±0.59
Autumn	147	235 23.5±2.9	1.59	70 (29.78%) 7±0.77^{a}	52 (22.13%) 5.2±0.72^{a}	59 (25.11%) 5.9±1.06	54 (22.98%) 5.4±0.68	44.26^{a} 5.4±0.82
Winter	197	279 23.22±1.89	1.42	100 (35.75%) 8.3±1.29^{b}	86 (30.88%) 7.17±0.89^{ab}	45 (16.15%) 3.75±0.86	48 (17.22%) 4±0.81	59.67^{b} 9.25±0.97
Spring	258	350 29.14±5.56	1.36	113 (32. 32%) 9.41±1.32^{b}	126 (36%) 10.5±2.08^{b}	71 (20. 25%) 5.9 ± 1.74	40 (11. 43%) 3.33±1.04	48.54^{b} 9.67±1.34

Values with different superscripts in the same column are significantly different at P<0.05.

Table (2): Effect of follicular diameter on quality of oocytes and maturation of buffalo oocytes (mean ±SEM).

Follicle Size (mm)	No. of follicles	oocyte number (total) mean±SE	Oocyte quality				Maturation rate
			Grade I Good% Mean±SE	Grade II Fair% Mean±SE	Grade III Poor% Mean±SE	Grade IV Denuded% Mean±SE	
Small sized (≤3)mm	42 7±1.15^{b}	31 5.17±0.7^{b}	32.3% 1.67±0.2^{b}	32.3% 1.67±0.33^{b}	19.34% 1±0.36^{ab}	16.06% 0.83±0.4^{ab}	35.14% 1.17±0.3^{b}
Medium sized (3-6)mm	86 14.3±1.74^{a}	72 12±1.46^{a}	34.75 % 4.17±0.6^{a}	27.75% 3.33±0.71^{a}	22.25% 2.67±0.67^{a}	15.25% 1.83±0.17^{a}	66.8% 3±0.52^{a}
Large sized (>6)mm	23 3.8±0.87^{b}	17 2.67±0.7^{b}	56.19% 1.5±0.42^{b}	37.45% 1.0 ±0.36^{b}	0.37% 0.01±0.34^{b}	5.99% 0.16±0.16^{b}	40% 1.67±0.56^{b}

Values with different superscripts in the same column are significantly different at (P<0.05).

Table (3): The effect of the CL on the ovarian surface on the oocytes yield, quality and maturation

Ovarian structure	No. of Ovaries in (Total) Mean±SE	oocyte number (total) mean±SE	No. of oocytes/ ovary (yield)	Oocyte quality				Maturation rate
				Grade I Good % (mean±SE)	Grade II Fair % (mean±SE)	Grade III Poor % (mean±SE)	Grade IV Denuded % mean±SE	
Ovary with CL	111 5.8±0.69	149 7.83±0.88	1.34	25.54 (2±0.42^{a})	25.54 (2±0.36^{a})	21.46 (1.68±0.29^{a})	27.46 (2.15±0.28^{a})	42 1.68±0.3^{a}
Ovary without CL	131 6.89 ±0.58	284 14.84±2.1	2.16	37.06 (5.5±0.7^{b})	34.7 (5.15±0.9^{b})	15.5 (2.3±0.35^{b})	12.74 (1.89±0.28^{b})	55.6 5.9±1.3^{b}

Values with different superscripts in the same column are significantly different at (P<0.05).

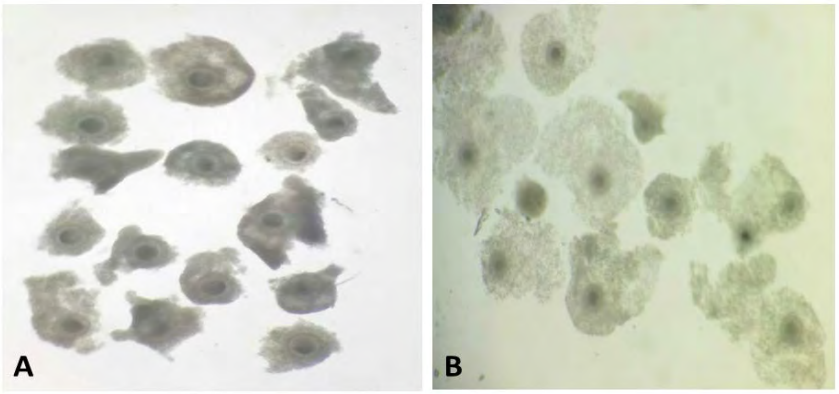

Fig. (1): Buffalo cumulus oocytes complexes (COCs). (A) Selected oocytes before maturation (B) matured oocytes after incubation 24 hr in 38.5 C (cumulus cell expansion).

4. DISCUSSION

The reproductive efficiency in buffaloes can be enhanced by using assisted reproductive technology (Madan *et al.*, 1994). The quality of oocytes have many challenges and was affected by different factors which decrease the efficiency of in vitro embryo production (IVEP) like ovarian morphology (Gandolfi *et al.*, 1998), age of oocytes (Yamamoto *et al.*, 2010), the stage of estrus cycle, diameter of follicles (Wurth *et al.*, 1994) and hormone concentration (Kruip and Dieleman, 1982).

The oocyte quality plays a main role in the assessment of oocyte developmental competence *in vitro* (Amer *et al.*, 2008). In the current study the number of oocytes recovered per ovary was (1.59) in the autumn and was close to that reported for Egyptian buffaloes (2.5) (Barkawi *et al.*, 2007) but less than that recorded for cattle (10 oocyte per ovary) (Gandolfi *et al.*, 1997). This might be attributed to low number of primordial follicles (10,000 to 19,000) in buffalo ovary compared to cattle (150,000) (Danell, 1987) which lead to low number of recovered oocytes in buffalo (Palta and Chauhan, 1998).

In the current study the oocyte yielding, quality and maturation rate were significantly ($P<0.05$) higher during the season associated with ovarian activity (winter and spring) than those harvested during the low breeding season (summer). A similar trend was reported by Seham *et al.* (2016), where the highest ($P<0.01$) incidence of ovarian activity was recorded during winter. The breeding efficiency in buffalo can be affected by ambient temperature, stress and relative humidity which also have a direct effect on *in vitro* maturation of oocytes (Leibfried-Rutledge *et al.*, 1989; Marai and Habeeb, 2010). There was a strong relation between season, oocytes quality, cumulus expansion, maturation and developmental competence of *in vitro* maturation of buffalo oocytes (Zoheir *et al.*, 2007; El-Naby *et al.*, 2013). The summer season in the present study had a higher number of bad quality degenerated oocytes. These findings are in agreement with results recorded by (Zeron *et al.*, 2001) who reported that the heat stress during summer season could change phospholipids composition of oocytes. In addition, the summer season affect reproduction in buffaloes by depressing the secretion of gonadotrophins as a result of hyper-prolactinaemia, which leads to change in ovarian steroidogenesis (Razdan *et al.* 1981). In contrast, Kadoom (1995) revealed that the quality of oocytes was increased in summer season. We supposed that heat stress produced during summer also affects folliculogenesis, follicular fluid microenvironment and oocyte quality. Buffaloes with summer anoestrus failed to exhibit oestrus as a result of aberration in the endocrine profile leading to ovarian inactivity or show silent heat (Danell, 1987).

Recently, the buffalo follicles and oocytes can express factors like Brain-derived neurotrophic factor (BDNF) throughout ovarian follicle development which can be assist the oocyte maturation and early embryogenesis (Zhao *et al.*, 2019). The diameters of follicles increased, and quality of oocytes become higher with multi- cumulus cells after stimulation of ovaries by FSH followed by GnRH (Sakaguchi *et al.*, 2019). The follicle size can deeply affect the quality

of oocytes obtained during ovulation (Sirard *et al.*, 2006). In the current study ovaries containing follicular diameter (3-6) mm were having good quality oocytes (grade I and II) ($P<0.05$) than small and large sized follicles. In accordance with the results of Raghu *et al.*, (2002) and Amer *et al.*, 2008), granulosa cells play an essential role in maturation and blastocyst stage development *in vitro* (Tanghe *et al.*, 2002). In addition, buffalo oocytes recovered from follicles ranged from (4-<6 and 6-<8mm) reached the M-II (67.1% and 79.1% respectively) than (2-<3 and 3-<4mm) (Yousaf and Chohan, 2003). However, oocytes from follicles (>4mm) were revealed to having good results in IVF in buffalo (Yousaf and Chohan, 2003. In contrast, increased fertilization, cleavage and embryo development were significantly higher in COCs more than 145 microm which can be aspirated from large follicles (> 8 mm) followed by medium and small-sized normal follicles (Raghu *et al.*, 2002). The obtained results revealed that oocytes harvested from medium sized follicle (3-6 mm) induced the best developmental competence in buffalo.

In the current study the ovaries without CL recorded a higher value for oocyte yield, quality (I, II, III) and maturation rate than ovaries with mature CL3. In accordance with results obtained by (Jamil *et al.* (2008) and Raza *et al.*, 2001). Kumar *et al.* (1997) reported that the presence of CL can restrict the follicular development. In addition, the ovaries bearing CL had large dominant follicles and the rest follicles were very small and hard to be harvested (Gasparrini *et al.*, 2000). Here, we proposed that ovaries without CL contained large number of high-quality follicles (medium sized), thereby oocytes yield, quality and maturation rate were optimum compared to ovaries with mature CL3. In contrast to these results, (Tasripoo *et al.*, 2005) recorded that oocytes harvested from ovaries containing active structure (dominant follicle or CL) had a higher maturation rate. However, Savio *et al.* (1988) examined buffalo ovaries and revealed that ovaries with CL contain more follicles and corpus luteum enhanced the follicular activity. The difference may be due to animal age, season, type of CL and nutritional state of animal.

CONCLUSION

The best results for IVM were obtained during winter and spring. Moreover, Egyptian buffalo oocytes recovered from medium sized follicles and ovaries without CL revealed maximum yield, quality and upgraded maturation *in vitro*.

REFERANCES

Aksu, E. H., Kandem, F.M., Kilic, K., Akman, O., Ömür, A. D. and Uçar, Ö., 2015. Arginase activity of ovarian structures in cows of Brown Swiss and its crossbreeds. Vet. Arhiv., 85:261-271.

Ali, A., Abdel-Razek, A.Kh. , Derar, R., Abdel-Rheem, H.A. and Shehata, S.H., 2009. Forms of reproductive disorders in cattle and buffaloes in Middle Egypt. Reprod. Domest. Anim., 44(4):580-6.

Amer, H.A., Hegab, A.O., and Zaabal, S.M., 2008. Effects of ovarian morphology on oocyte quantity and quality, granulosa cells, *in vitro* maturation and steroid hormone production in buffaloes. Anim., Reprod., 5: 55-62.

Barkawi, A. H., Ibrahim, S. A., Ashour, G., El-Asheeri, A. K., Hafez, Y. M. and Faheem, M.S., 2007. In vitro production of Buffalo (Bubalus bubalis) embryos. Egyptian J. Anim. Prod., 44(1):35-48

Bavister, B. and Rose-Hellekant, T., 1992. Development of *in vitro* matured/*in vitro* fertilized bovine embryos into morulae and blastocysts in defined culture media. Theriogenology, 37:127-146.

Chohan, K., and Hunter, A., 2003. In vitro maturation and fertilization of water buffalo oocytes. Buffalo J., 19:91-101.

Danell, B., 1987. Oestrus Behavior, Ovarian Morphology and Cyclical Variation in the Follicular System and Endocrine Pattern in Water Buffalo Heifers. Uppsala, Sweden: Swedish University of Agricultural Sciences. Thesis.

El-Naby, Al. H., Mahmoud, K.G.H.M., Ahmed, Y.F., Abouel-Roos, M. E.A. and Ghaffar, A. E., 2013. Effect of season of the year and ovarian structures on oocytes recovery rate, quality and meiotic competence in Egyptian buffaloes. Global Veterinaria, 10: 408-412.

Gad, A., Abu Hamed, S., Khalifa, M., Amin, A., El-Sayed, A., Swiefy, S.A. and El-Assal, S., 2018. Retinoic acid improves maturation rate and upregulates the expression of antioxidant-related genes in *in vitro* matured buffalo (*Bubalus bubalis*) oocytes. Int. J. Vet. Sci. Med., 6(2):279-285.

Gandolfi, F., Luciano, A. M., Modina, S., Ponzini, A., Pocar, P., Armstrong, D. T., and Lauria, A., 1997. The *in vitro* developmental competence of bovine oocytes can be related to the morphology of the ovary. Theriogenology, 48:1153-1160.

Gandolfi, F., Milanesi, E., Pocar, P., Luciano, A.M., Brevini, T.A.L., Acocella, F., Lauria, A. and Armstrong, D.T., 1998. Comparative analysis of calf and cow oocytes during in vitro maturation. Mol. Reprod. Dev., 49:168-175.

Gasparrini, B., Neglia, G., Di Palo, R., Campanile, G. and Zicarelli, L., 2000. Effect of cysteamine during *in vitro* maturation on buffalo embryo development. Theriogenology, 54: 1537-1542.

Haldar, A. and Prakash, B.S., 2007. Effect of exogenous growth-hormone-releasing factor on blood metabolites and minerals in late maturing buffalo heifers (Bubalus bubalis). J. Anim. Physiol. Anim. Nutr., 91:326-332.

Hosseini, S.M., Moulavi, F., Hajian, M., Abedi, P., Forouzanfar, M. and Ostad Hosseini, S., 2008. Highly efficient *in vitro* production of bovine blastocyst in cell-free sequential oviductal fluid vs. TCM199 Vero cell co-culture system. Int. J. of Ferti. and Steri., 2: 66-73.

Hussein, T.S., Thompson, J.G. and Gilchrist, R.G., 2006. Oocyte-secreted factors enhance oocyte developmental competence. Dev. Biol, 296: 514-521.

Jamil, H., Samad, H.A., Qureshi, Z.I., Rehman, N. U. and Lodhi, L.A., 2008. Harvesting and Evaluation of Riverine Buffalo Follicular Oocytes. Turk. J. Vet. Anim. Sci., 32: 25-30.

Kadoom, A. Kh. A., 1995. Studies on: in vitro maturation, fertilization and development of buffalo oocytes. Ph.D. Thesis (Gynecology, Obstetrics and A.I. Faculty of Vet. Med. Alex., University.

Kruip, T.A.M. and Dieleman, S.J., 1982. Macroscopic classification of bovine follicles and its validation by micromorphological and steroid biochemical procedures. Reprod. Nutr. Dev., 22:465-473.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N., and Jain, G.G., 1997. Oocyte retrieval and histological studies of follicular population in buffalo ovaries. Anim. Reprod. Sci., 47: 189-195.

Lee, E.S., and Fukui, Y., 1995. Effect of various growth factors in a defined culture medium on in vitro development of bovine embryos matured and fertilized in vitro. Theriogenology, 44:71-83.

Leibfried, L., and First, N.L., 1979. Characterization of bovine follicular oocytes and their ability to mature in vitro. J. Anim. Sci, 48:76-86.

Leibfried-Rutledge, M., Crister, E. S., Parrish, J. J. and First, N. L., 1989. *in vitro* maturation and fertilization of bovine oocytes. Theriogenology, 31: 61-74.

Madan, M.L., Chauhan, M.S., Singla, S.K. and Manik, R.S., 1994. Pregnancies established from water buffalo (Bubalus bubalis) blastocysts derived from in vitro matured in vitro fertilized oocytes and co-cultured with cumulus and oviductal cells. Theriogenology, 42:591- 600.

Marai, I.F.M. and Habeeb, A.A.M., 2010. Buffaloe's reproductive and productive traits as affected by heat stress. Tropical and Subtropical Agroecosystems, 12: 193 - 217.

Nandi, S., Chauhan, M. and Palta, P., 2001. Effect of environmental temperature on quality and developmental competence of buffalo oocytes. *Vet Rec,* 148:278-279.

Nandi, S., Ravindranatha, B.M., Gupta, P.S.P. and Sarma, P.V., 2002. Timing in sequential changes in cumulus cells and first polar body extrusion during in vitro maturation of buffalo oocytes. Theriogenology, 57:1151-1159.

Palta, P. and Chauhan, M. S., 1998. Laboratory production of buffalo (Bubalus bublis) embryos. Reprod. and Ferti. Dev., 10:379 - 391.

Raghu, H., Nandi, S. and Reddy, S., 2002. Follicle size and oocyte diameter in relation to developmental competence of buffalo oocytes in vitro. Reprod. Fertil Dev., 14:55-61.

Raza, A., Samad, H.A., Rehman, N.U. and Zia, E.U.H., 2001. Studies on *in vitro* maturation and fertilization of Nili-Ravi buffalo follicular oocytes. Int. J. Agri.Biol., 3: 503-506.

Razdan, M.N., Kakar, M.L. and Galhotra, M.M., 1981. Serum luteinizing hormone levels of non-cycling buffaloes (Bubalus bubalis). Indian J. Anim. Sci., 51, 286–288.

Ribeiro, H.F.L., Vale, W.G., Andrade, V.J. and Marques, A.P., 2003. Environmental effect on the ovarian post-partum activity in the buffaloes raised in low Amazon region, Brazil. Buffalo J. 3, 311‑321.

Sakaguchi, K., Maylem, E.R.S., Tilwani, R.C., Yanagawa, Y., Katagiri, S., Atabay, E.C., Atabay, E.P. and Nagano, M., 2019. Effects of follicle-stimulating hormone followed by gonadotropin-releasing hormone on embryo production by ovum pick-up and in vitro fertilization in the river buffalo (Bubalus bubalis). Anim Sci J. doi: 10.1111/asj.13196.

Savio, J.D., Keenan, L., Boland, M.P. and Roche, J.F., 1988. Pattern of growth of dominant follicles during the oestrous cycle of heifers. J. Reprod. Fertil., 83: 663-671

Seham, S. S., Mahmoud, Z., Attia, A.S., Abdoon, A.S.S., Nahed, E.E., Omaima, M. K., and Hussein, A. S., 2016. Seasonal variation in ovarian functions in Egyptian buffalo and cattle. Int. J. Pharm. Tech., 9(6):34-42.

Singh, J. and Adams, G.P., 2000. Histomorphometry of dominant and subordinate bovine ovarian follicles. Anat. Rec., 258:58-70.

Sirard, M.A., Richard, F., Blondin, P., and Robert, C., 2006. Contribution of the oocyte to embryo quality. Theriogenology, 65(1): 126-136.

Tasripoo, K., Srisakwattana, K., Suthikrai, W., Chethasing, S. and Kamonpatana, M., 2005. Potential uses of buffalo oocytes from ovaries with CL and without CL for in vitro maturation and fertilization. Buffalo J., 21 (3): 221-228.

Tanghe, S., Soom, A., Nauwynck, H., Coryn, M. and DeKruif, A. 2002. Mini review: functions of the cumulus oophorus during oocyte maturation, ovulation and fertilization. Mol. Reprod. Dev., 61:414-424.

Wurth, Y.A., Boni, R., Hulshof, S.C.J. and Kruip, T.A.M., 1994. Bovine embryo production in vitro after selection of ovaries, follicles and oocytes. In: Wurth YA. Bovine Embryo Production In Vitro: Influencing factors. Utrecht, The Netherlands: Utrecht University Press. 67-85.

Yamamoto, T., Iwata, H., Goto, H., Shiratuki, S., Tanaka, H., Monji, Y. and Kuwayama, T., 2010. Effect of maternal age on the developmental competence and progression of nuclear maturation in bovine oocytes. Mol. Reprod. Dev., 77:595-604.

Yousaf, M.R., Chohan, K.R., 2003.Nuclear morphology, diameter and meiotic competence of buffalo oocytes relative to follicle size. Reprod Fertil Dev., 15(4):223-9.

Zhao, X., Du, F., Liu, X., Ruan, Q., Wu, Z., Lei, C., Deng, Y., Luo, C., Jiang, J. and Shi, D., Lu, F., 2019. Brain-derived neurotrophic factor (BDNF) is expressed in buffalo (Bubalus bubalis) ovarian follicles and promotes oocyte maturation and early embryonic development. Theriogenology, 130:79-88.

Zoheir, K.M.A., Abdoon, A. S., Mahrous, K. F., Amer, M. A., Zaher, M. M., Li-Guo, Y. and El- Nahass, E. M., 2007. Effects of season on the quality and *in vitro* maturation rate of Egyptian buffalo (Bubalus bubalis) oocytes. J. Cell and Anim. Biol., 1(2): 029-033.

Zeron, Y., Ocheretny, A., Kedar, O., Borochov, A., Sklan, D. and Arav, A., 2001. Seasonal changes in bovine fertility: Relation to developmental competence of oocytes, membrane properties and fatty acid composition of follicles. Reprod., 121: 447-454.