



Effect of Seasons on Ovarian Activity and Developmental Competence of Buffalo Oocytes and Embryos



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THE AIMS of the current study were to investigate the effects of seasons on 1) oocyte yield, aspiration rate, and quality in buffalo. 2) Maturation rate and embryo developmental competence in buffalo. Buffalo ovaries [426] were collected during different seasons of the year [2021], cold seasons (from October to March) and hot seasons (from April to September), and the temperature humidity index (THI) was calculated, counting the number of ovarian follicles, and the aspiration rate and yield were recorded. Oocytes' quality was classified as excellent, good, fair, and denuded. Excellent and Good quality oocytes were *in vitro* matured (IVM) in tissue culture media -199 (TCM-199) + fetal calf serum 10% (FCS) + 10 µg/ml follicle-stimulating hormone (FSH) + 50 µg/ml gentamicin. Oocytes were incubated for 22 h in 5% CO₂ and 38.5°C., cumulus-cell expansion and nuclear maturation were determined. Frozen-thawed semen was used to fertilize mature oocytes, which were then incubated for 18 hours before being cultured *in vitro* for 7 days by synthetic oviduct fluid (SOF). Results demonstrated that the total and mean number of buffalo oocytes yield in the cold seasons was significantly (P<0.05) higher when compared to hot seasons. Aspiration rate is not significantly affected by season. Cold seasons are characterized by an increased number and percentage of excellent and good oocytes Also, the hot seasons significantly (P<0.05) decreased cumulus-cell expansion and nuclear maturation of IVM buffalo oocytes. The transferable embryo rate was significantly (P<0.05) higher in cold seasons when compared with hot seasons. In Conclusion, the hot seasons could impair reproduction in buffalo by decreasing the oocyte yield and quality. *In vitro* maturation and transferable embryo rates significantly increased during the cold seasons in Buffalo.

Keywords: Season, yield, Oocyte quality, *In vitro* maturation, Embryo development, Buffalo.

Introduction

Nowadays, Global warming may be a common problem touching largely the livestock population and inflicting severe economic losses worldwide. In many countries, including Egypt, the agricultural economy depends heavily on buffaloes. They

are used for supplying meat and milk. However, reproductive performance in buffalo is low compared to cattle. Delayed puberty, silent estrus, low conception rate, and long inter-calving interval are among the foremost factors affecting reproduction in buffalo. Buffaloes are sensitive to heat stress (HS) during summer due to their

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(Received 28/02/2023, accepted 18/05/2023)

DOI: 10.21608/EJVS.2023.196836.1451

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specific peculiarities such as the black coloration of the skin and the low number of sweat glands.

HS was reported to decrease the number of antral follicles and good-quality oocytes in buffalo ovaries [1]. Additionally, buffalo oocytes possess more lipids than many other species and they are particularly vulnerable to oxidative stress (OS), which impairs the oocyte quality and affects their reproductive potential [2]. During hot seasons, HS induces an increase in reactive oxygen species (ROS) levels which in turn have a role in the detrimental effects on the integrity and functions of gametes [3]. Increased ROS levels above the physiological range may result in OS and can worsen the oocyte quality and subsequently influence reproductive outcomes [4]. The majority of goat oocytes that were cultured under heat-shock circumstances stayed at the germinal vesicle breakdown (GVBD) stage [5], and they displayed an aberrant chromatin configuration caused by OS in the oviduct, which may be related to heat stress-induced early embryonic death [6]. Also, HS impairs the developmental competence of buffalo oocytes through the alteration of heat shock protein 70 mRNA gene expression [7].

In the last few decades, there has been a surge in interest in *in vitro* embryo production in buffalo at a global scale to improve genetic progress [8]. However, there are still restrictions to this technology, such as the innate lower number of follicles and thus COCs recovered per ovary [9], in addition to seasonality [10].

Consequently, the current study was conducted to evaluate the effect of seasons on oocyte yield, aspiration rate, oocyte quality, and the rate of maturation and embryo developmental competence in buffalo.

Material and Methods

The current investigation was conducted at Egypt's National Research Center's "Embryo and Genetic Resources Conservation Bank."

Temperature humidity index (THI) was calculated according to the formula proposed by Mader et al. [11]:

$$\text{Temperature-humidity index (THI)} = 0.8 \times \text{ambient temperature} + [(\% \text{ relative humidity}) 100 \times (\text{ambient temperature} - 14.4)] + 46$$

TABLE 1. Mean Monthly Temperatures and Humidity Conditions in Qalyubia Governorate in Egypt for the Study Period [2021].

Month	Average temperature	Maximum temperature	Minimum temperature	Relative Humidity %	THI
January	16.8	21.5	12.5	61.5	55.772
February	16.4	22.1	11.8	59	54.796
March	18.3	23.9	13	49.6	55.7168
April	21.1	28.6	14.3	42.5	57.8475
May	28	35.4	20.5	36.7	64.676
June	27.7	34.1	21.7	46.3	66.9851
July	30.8	36.7	25.3	49.7	71.9476
August	31.8	37.4	26.6	51.6	73.8488
September	27.7	33	23	54.2	69.1734
October	24.5	29.4	20.3	56.7	65.4915
November	22	26.6	17.8	60.5	62.91
December	16.1	19.5	12.7	58.6	54.3146

THI= temperature humidity index.

Unless otherwise stated, all of the compounds used in this investigation were bought from Sigma-Aldrich. The ovaries of the buffalo were collected from Awlad Bakry slaughterhouse in Qalyubia Governorate in Egypt and transferred to the lab in a tank filled with normal saline solution (NSS, 0.9% NaCl with 100 µg/ml streptomycin and 100 IU penicillin). Ovaries were cleaned in the lab many times in pre-heated NSS (37°C) and then maintained in the water bath at 37°C until aspiration. Using an 18-gauge needle connected to a 10 ml sterile syringe containing 2 ml of aspiration washing medium (phosphate buffered saline; PBS) + 6 mg/ml bovine serum albumin F-V + 50 µg/ml gentamicin, COCs were aspirated from follicles with a diameter of 2 to 8 mm. Following aspiration, follicular fluid was placed in a Falcon tube and left to settle down for 15 min at 37°C water bath. COCs were examined by a stereo microscope at a magnification of 90 and washed three times in an aspiration medium.

Experimental Design:

Effect of Seasons on Yield, Aspiration Rate, and Quality of Oocytes in Buffaloes

A total number of 426 ovaries were collected during this experiment, during cold seasons (from October to March) (Autumn and Winter) [178] and hot seasons (from April to September) (Spring and Summer) [248] through 10 replicates and temperature humidity index (THI) was calculated as mentioned before.

The number of recovered oocytes was counted per replicate under a stereomicroscope at 90X. The oocyte yield was estimated by the following equation:

$$\text{Oocyte yield} = \frac{\text{Total No. of recovered oocytes}}{\text{Total No. of ovaries per trial}}$$

The mean number of oocytes was calculated:

$$\text{The mean number of oocytes} = \frac{\text{No. of oocytes}}{\text{No. of replicates}}$$

The aspiration rate (Recovery rate) of oocytes was estimated as follows:

$$\text{Aspiration rate (Recovery rate)} = \frac{\text{No. of oocytes}}{\text{No. of follicles}} \times 100$$

According to Kandil et al. [12], the quality of buffalo oocytes (COCs) was assessed. Depending on their cumulus investment and evenly granulated ooplasm, oocytes were divided into 4 groups

under a stereomicroscope [90 x] as follows:

Excellent: oocytes with at least five layers of fully developed cumulus cells and evenly granulated dark cytoplasm.

Good: one to four layers of cumulus cells and evenly granulated dark cytoplasm.

Fair: Oocytes are incompletely encircled by cumulus cells, and the ooplasm has little granulation.

Denuded: oocytes were covered by zona pellucida and had no cumulus cells.

Percentage of oocyte quality=

$$\frac{\text{Number of COCs per grade}}{\text{The total No. of oocytes recovered}} \times 100$$

Effect of Seasons on Oocyte Maturation Rates in Buffalo

Good-quality oocytes (excellent & good) were washed three times in TCM-199 medium and then cultured in TCM-199 enriched with 10% FCS + 10 µg/ml FSH + 50 µg/ml gentamicin. Maturation of the oocytes was done in the incubator for 22 h in a humidified environment with 5% CO₂ and 38.5°C.

According to the extent of cumulus-cell development, the cytoplasmic maturation of buffalo oocytes was evaluated and divided into 4 grades (G 0, GI, GII, GIII) [12].

G0: without expansion. **GI:** with slight expansion. **GII:** with moderate expansion

GIII: with complete expansion

The expansion rate was evaluated according to the following equation:

$$\text{Expansion rate} = \frac{\text{No. of oocytes per grade}}{\text{Total No. of oocytes}} \times 100$$

The oocytes' nuclear maturation was estimated by the presence of the 1st polar body (Pb) in perivitelline space. To achieve this, at the end of the maturation period, decumulation of oocytes (removal of cumulus- cells by repeated gentle pipetting by a 100 µl pipette) was done then the number of oocytes that have first Pb was detected.

The nuclear maturation rate (MII) was detected according to the following equation:

$$\text{M II} = \frac{\text{No. of mature oocytes with 1st Pb}}{\text{Total No. of oocytes}} \times 100$$

Effect of Seasons on Embryo Developmental Competence in Buffaloes

Matured oocytes that had fully expanded cumulus cells and the first Pb were washed in a fertilization medium (Fert-TALP enriched with 6 mg/l BSA). In a water bath set at 37°C, Frozen sperm were thawed for 30 seconds. Frozen thawed semen was placed on the top of 2 layers of percoll density gradient (90% and 45%) and centrifuged for thirty minutes at 1800 rpm. The percoll & supernatant were discarded, and the semen pellet was mixed with 5 ml sperm-TALP medium containing 10 µg/ml heparin and 4 mg/ml BSA, then centrifugation once more for ten minutes at 1800 rpm. The supernatant was discarded, and the semen pellet was re-suspended in Fert- TALP medium enriched with 10 µM/ml hypotaurine, 20 µM penicillamine (PHE) + 1µg/ml heparin and 6 mg/ml BSA. The concentration of the perm was elevated to 1×10⁶ sperm/ml before being placed in a four-well culture plate. The sperm and oocytes were incubated together for eighteen h at 38.5°C with 5% CO₂ in a humid atmosphere. After at least three washings, the presumptive zygotes were cultured in culture medium (IVC, mSOFaa medium) enriched with 5 mg/ml BSA, 5 µg/ml insulin, and 50 µg /ml gentamycin and incubated at 38.5°C with 5% CO₂. On Days 2, 5, and 7, the hatching rate and embryo development to the morula, and blastocyst stages (transferable embryos) were assessed. The culture medium was replaced every 48 h.

Detection of the cleavage rate and transferable embryos in the cold season group vs. the hot season's group were detected according to the following:

$$\text{Cleavage rate} = \frac{\text{No. of cleaved oocytes}}{\text{Total No. of matured oocytes}} \times 100$$

The developmental stage rate =

$$\frac{\text{No. of the developmental stage}}{\text{Total No. of the cleaved}} \times 100$$

Statistical Analysis

All data were expressed as the mean ± SEM and the significant differences were evaluated using paired t-test. Statistical analyses were performed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA).

Results

Effect of Seasons on Oocytes Yield and Aspiration Rate in Buffalo

The ovaries were collected in cold seasons [n=178] with a mean of 17.80±1.05 where the temperature humidity index (THI) mean was 58.94±2.13 and hot seasons (n=248) with a mean of 24.80± 1.84 where the temperature humidity index (THI) mean was 67.51±2.90 (Table 2). The total and mean number of buffalo follicles in cold seasons were n=766, 76.60±4.60, respectively, and in hot seasons were n=1043, 104.30±8.16, respectively. The number of oocytes aspirated from follicles was 574 in cold seasons and 660 in hot seasons with the mean of oocytes/replicates being 57.40±3.40 and 66.00±5.51 in cold seasons and hot seasons respectively. The mean number of buffalo oocytes yield/ovary in the cold seasons (3.22±0.028) was significantly (P<0.05) higher when compared to the hot seasons (2.64±0.093 oocyte/ovary). The aspiration rate of oocytes from follicles in cold seasons (75.76±3.65%) and

TABLE 2. Effect of Seasons on Oocytes Yield and Aspiration Rate in Buffalo

Season	No. of ovaries		No. of Follicles		No. of oocytes		Yield	Aspiration rate
	No.	*Mean± S.E.	No.	*Mean±S.E	No.	*Mean ± S.E.	*Mean ± S.E.	%
Cold(THI=58.94)	178	17.80±1.05	766	76.60±4.60	574	57.40±3.40	3.22±0.028 ^a	75.76±3.65 ^a
Hot(THI =67.51)	248	24.80±1.84	1043	104.30±8.16	660	66.00±5.51	2.64±0.093 ^b	63.10±1.61 ^a

*Replicates = 10

Superscripts a and b will be statistically compared inside the same column. The values with different letter superscripts differ significantly (P<0.05).

hot seasons ($63.10 \pm 1.61\%$) showed no significant difference.

Effect of Seasons on Oocytes Quality in Buffalo

Excellent and good quality oocytes percentage collected during cold seasons (Table 3, Figs.1 A) showed a higher significance ($P < 0.05$) [39.83 %, and 32.19 %, respectively] than those collected during the hot seasons season (25.45, 19.20 %, respectively).

The hot seasons season showed greater significant ($P < 0.05$) values of low-quality oocytes (fair and denuded) [17.0 ± 1.13 , 26.40 % and 19.20 ± 3.85 , 28.38%, respectively] than oocytes mean and percentage during the cold seasons season (7.20 ± 1.27 , 13.04 % and 8.30 ± 1.24 , 14.91 %, respectively). The cold seasons showed higher-excellent and good oocytes quality in comparison with fair and denuded oocytes quality.

Effect of Seasons on Cumulus Expansion of Buffalo Oocytes

The cold seasons had higher significant ($P < 0.05$) values of GIII and GII cumulus expansion mean and percentage (16.40 ± 1.90 ; 38.60 ± 1.45 %, respectively) and (14.40 ± 1.66 , 34.20 ± 1.44 %, respectively) when compared to the hot seasons (6.40 ± 0.71 , 22.22 ± 1.02 %, respectively) and (6.70 ± 0.84 , 22.40 ± 1.06 %, respectively) (Table 4, F,1B).

G0 cumulus expansion had a higher significant ($P < 0.05$) mean and percentage in the hot seasons [8.00 ± 1.15 , 26.47 %] than in the cold seasons [5.80 ± 0.55 , 14.32%]. The GI cumulus expansion showed no significant difference in mean between

cold seasons (5.30 ± 0.57) and hot seasons [8.90 ± 1.02] but there was a greater significance ($P < 0.05$) percentage in hot seasons than cold seasons [30.32 % and 12.86%, respectively].

Effect of Season on Nuclear Maturation Rate (1st polar body) of Buffalo Oocytes.

The average number of mature buffalo oocytes with the Pb (Table 5, Fig. 1C) cultured in TCM-199 in cold seasons and hot seasons (mean \pm S.E) was (32.60 ± 3.25) and (20.50 ± 2.50), respectively. The average number of respective matured oocytes without polar bodies was (9.30 ± 1.08) and (9.30 ± 1.22).

The percentage of buffalo oocytes that have polar bodies was significantly ($P < 0.05$) greater in the cold seasons [$77.94 \pm 1.02\%$] when compared with those matured in hot seasons (69.15 ± 0.98 %). The buffalo oocytes without polar bodies matured in hot seasons revealed a significant ($P < 0.05$) difference ($30.84 \pm 0.98\%$) when compared with cold seasons (22.05 ± 1.02 %).

Effect of Season on Developmental Competence of Buffalo Embryos

The cold seasons showed higher significant ($P < 0.05$) values of morula and blastocyst mean and percentage (4.20 ± 0.51 , 16.42 ± 0.88 %, respectively) and (3.0 ± 0.36 , 11.60 ± 0.60 %, respectively) when compared to the hot seasons (1.20 ± 0.20 , 8.41 ± 1.31 %, respectively) and (91.0 ± 0.21 , 6.43 ± 1.30 %), respectively.

The embryo cleavage rate (Table 6, Fig, 1D, E) was significantly ($P < 0.05$) greater in cold seasons [35.40 ± 3.02] when compared with hot

TABLE 3. Effect of Season on Oocytes Quality in Buffalo

Seasons	No. of ovaries		No. of Oocytes		Excellent		Good		Fair		Denuded					
	No.	*Mean \pm S.E.	No.	*Mean \pm S.E.	No.	*Mean \pm S.E.	%	No.	*Mean \pm S.E.	%	No.	*Mean \pm S.E.				
Cold (THI = 58.94)	178	17.80 ± 1.05	574	57.40 ± 3.40	230	23.00 ± 2.31^a	39.83 ± 2.62^a	189	18.90 ± 2.49^a	32.19 ± 2.81^a	72	7.20 ± 1.27^b	13.04 ± 2.18^b	83	8.30 ± 1.24^b	14.91 ± 2.13^b
Hot (THI = 67.51)	248	24.80 ± 1.84	660	66.00 ± 5.51	166	16.60 ± 2.23^b	25.45 ± 3.10^b	132	13.20 ± 1.71^a	19.20 ± 1.04^b	170	17.00 ± 1.13^a	26.40 ± 1.45^a	192	19.20 ± 3.85^a	28.38 ± 4.27^a

*Replicates = 10

Superscripts a and b will be statistically compared inside the same column. The values with different letter superscripts differ significantly ($P < 0.05$).

TABLE 4. Effect of Season on Buffalo Oocytes Cytoplasmic Maturation Rate (cumulus expansion)

Seasons	No. of Ovaries	No. of Oocytes	GIII			GII			GI			G 0			
			No.	*Mean ± S.E.	%	No.	*Mean ± S.E.	%	No.	*Mean ± S.E.	%	No.	*Mean ± S.E.	%	
Cold (THI = 58.94)	178	419	41.90±	164	16.40 ±	38.60±	144	14.40±	34.20±	53	5.30 ±	12.86±	58	5.80	14.32±
			4.22		1.90 ^a	1.45 ^a		1.66 ^a	1.44 ^a		0.57 ^a	1.20 ^b		0.55± ^b	1.26 ^b
Hot (THI = 67.51)	248	298	29.80±	64	6.40	22.22±	67	6.70±	22.40±	89	8.90±	30.32±	80	8.00±	26.47±
			3.66		±0.71 ^b	1.02 ^b		0.84 ^b	1.06 ^b		1.02 ^a	1.14 ^a		1.15 ^a	1.08 ^a

*Replicates = 10

Superscripts a and b will be statistically compared inside the same column.

The values with different letter superscripts differ significantly (P<0.05).

TABLE 5. Effect of Season on Nuclear Maturation rate (1st polar body) of Buffalo Oocytes.

Seasons	No. of ovaries	No. of Oocytes		1 st pb			Without 1 st pb		
		No.	*Mean ± S.E.	No.	*Mean ± S.E.	%	No.	*Mean ± S.E.	%
Cold (THI = 58.94)	178	419	41.90±4.22	326	32.60±3.25 ^a	77.94±1.02 ^a	93	9.30±1.08 ^a	22.05±1.02 ^b
					Hot (THI = 67.51)	248		298	29.80±3.66

*Replicates = 10

Superscripts a and b will be statistically compared inside the same column.

The values with different letter superscripts differ significantly (P<0.05).

seasons (21.10±2.48). The 2-4 developmental stage rate showed a higher significant increase (P<0.05) [42.17±1.30 %] in the hot seasons when compared with the cold seasons (34.51±1.10 %). The 8-16 developmental stage revealed no significant difference in mean and rate in cold seasons (9.40±0.84, 37.39±1.50 %) when compared with hot seasons (6.0±0.69, 42.97±1.13 %), respectively.

Discussion

Effect of Seasons on Oocytes Yield and Aspiration Rate in Buffalo

Results obtained in the current study indicated that the season significantly affects the oocyte yield. The total and mean number of buffalo oocytes yield in the cold seasons (574, 3.22± 0.02 oocyte/ovary, respectively) were significantly (P<0.05) higher when related to the hot seasons (660, 2.64 ± 0.09 oocyte/ovary, respectively).

This data agreed with those conducted by Shukla *et al.* [13] who recorded that the number of buffalo oocytes retrieved per ovary was significantly greater during the winter (3.94±0.09) as compared to the summer (2.62 ± 0.06)season. Also, Soliman *et al.* [1], recorded a lower yield

TABLE 6. Effect of Season on Embryo Developmental Competence in Buffaloes:

Seasons	Ovaries No.	Oocytes No.	No. of mature oocytes		Cleavage rate		2-4 cell stage		8-16 cell stage		Morula		Blastocyst				
			No.	%	*Mean ± S.E.	No.	%	*Mean ± S.E.	No.	%	*Mean ± S.E.	No.	%	*Mean ± S.E.	No.	%	
Cold (TH = 58.94)	178	419	326	77.94 ± 3.25 ^a	25.40 ± 2.47 ^a	78.04 ± 0.63 ^a	88	8.80 ± 0.91 ^a	34.51 ± 1.10 ^b	94	9.40 ± 0.84 ^a	42	4.20 ± 0.51 ^a	16.42 ± 0.88 ^a	30	3.00 ± 0.36 ^a	11.6 ± 0.60 ^b
Hot (TH = 67.51)	248	298	205	69.15 ± 2.50 ^b	14.10 ± 1.66 ^b	69.36 ± 1.07 ^b	59	5.90 ± 0.72 ^a	42.17 ± 1.30 ^a	60	6.00 ± 0.69 ^a	12	1.20 ± 0.20 ^b	8.41 ± 1.31 ^b	10	1.00 ± 0.21 ^b	6.43 ± 1.30 ^b

Superscripts a and b will be statistically compared inside the same column. The values with different letter superscripts differ significantly (P<0.05).

*Replicates = 10

of oocytes per ovary in buffaloes during the hot season and the total number of oocytes recovered per ovary was significantly greater during cool months than during moderately hot and hot months. The former authors attributed the poor recovery of oocytes during hot months to the relatively inactive status of ovaries during that time. This may be attributed to the inhibition of the development of the ovarian follicle due to the hyperprolactinemia produced as a result of heat stress which in turn inhibits the secretion of both FSH and LH at the hypophyseal level [14]. Moreover, buffaloes are more sensitive to Heat stress (HS) throughout summer because of their specific peculiarities like the black coloration of the skin and also the low number of sweat glands.

On the contrary, Di Francesco et al. [15] added that heat stress has no impact on the number of buffalo oocytes recovered per ovary. These discrepancies could be related to the time, duration, or severity of heat stress during summer and differences in genetic susceptibility to HS.

The aspiration rate in the present study is higher in the cold season [75.76%] than in the hot season (63.1%) but without significance. This rate was higher than that indicated by Shukla et al. [13] (68.35) and Hammad et al. [16] (72.68%) in winter in buffalo and El-Moghazy et al. [17] in cattle (71.85%). The percentage of aspiration in the current study was also higher than those recorded by Shahid et al. [18] (47.78 to 55 %), Raziq et al. [19] (30.22%) in buffalo. The variation might be due to the difference in age of the animal, the climatic conditions, the breed, the ovarian status, and the ovarian environment at the time of collection, the functional status of follicles nutritional status, and the cyclicity of animals at the time of slaughter (20; 21; 22).

Effect of Season on Oocytes Quality in Buffalo

The data of the present study revealed higher percentage levels of excellent and good quality oocytes collected during the cold season (39.83, and 32.19 %, respectively) than those collected during the hot season (25.45, 19.20 %, respectively) while the hot season showed higher significant values of low quality (fair and denuded) [26.40, 28.38%, respectively] oocytes than oocytes percentage during the cold season (13.04, 14.91 %, respectively).

These results agreed with Mansor, [23] and Shukla et al. [13] who recorded that grade I (good) oocytes were markedly (P<0.05) greater

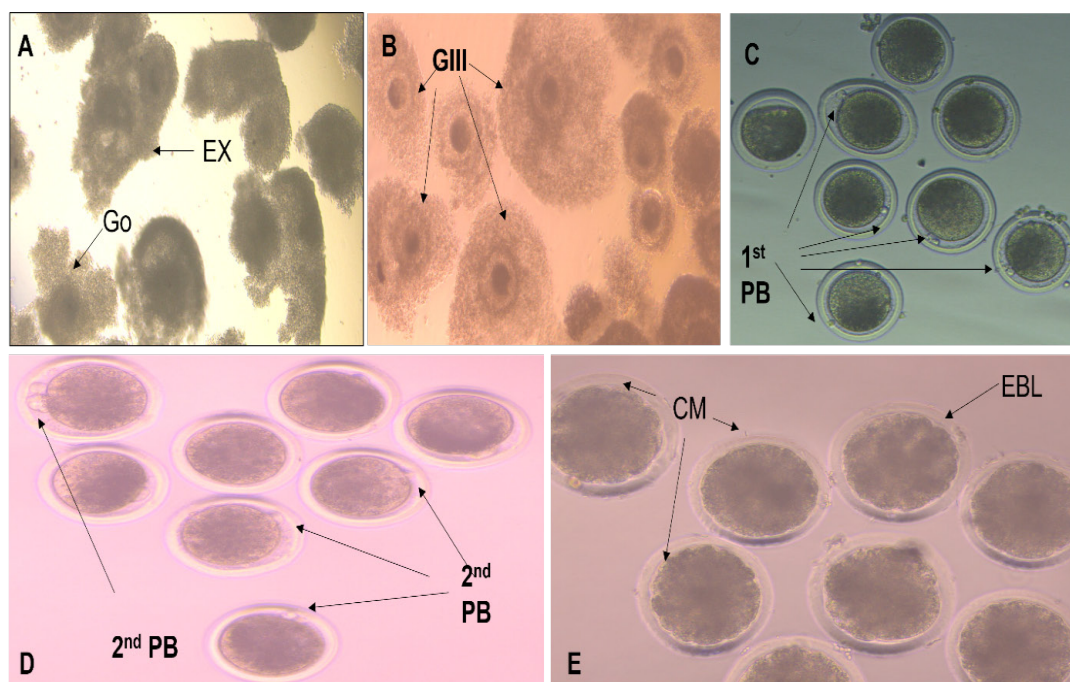


Fig. 1. Buffalo oocyte quality and development during cold seasons, A) oocyte quality Ex=Excellent oocyte, Go= Good oocyte, B) Cumulus expansion in matured oocytes GIII=full expansion of cumulus, C) Nuclear maturation of oocytes 1st PB= extrusion of 1st polar body, D) Fertilized oocytes 2nd PB= 2nd polar body, E) transferable embryo CM=compact morula, EBL= early blastocyst.

during winter and spring seasons in Buffalo. The percentages of excellent & good quality oocytes were significantly ($P < 0.01$) higher during winter and spring than in summer and autumn, while fair and denuded oocytes were significantly ($P < 0.01$) higher in summer and autumn than winter and spring in buffalo and cattle [1]. In contrast to our findings, Italian Mediterranean buffaloes did not exhibit a seasonal variation in the incidence of good-quality oocytes [8] which is probably related to the varied breeds and agro-climatic circumstances. Thermal seasonal stress can change the phospholipids content of oocytes which will impact the quality of oocytes [24].

Effect of Season on Oocyte Maturation Rate in Buffalo.

Our study indicated higher percentage levels of GIII and GII cumulus expansion in the cold seasons [38.60, 34.20% respectively] than in the hot seasons (22.22, 22.40 %, respectively) and higher nuclear maturation rate in the cold seasons (77.94%) than a hot season (69.15%).

These data are concomitant with those previously recorded by Abdoon *et al.* [7] who revealed that buffalo oocytes matured over the

cold season and displayed homogenous cytoplasm and healthy expanding cumulus cells. On the other hand, oocytes that matured during the hot season displayed dark cytoplasm and cumulus cell degeneration, and the MII of buffalo oocytes during the hot seasons significantly ($P < 0.01$) decreased and increased the percentage of oocyte degeneration. A significant increase ($P < 0.05$) was recorded in the maturation rate in autumn compared with spring, summer, and winter [25].

Moreover, the maturation rate of bovine oocytes was adversely affected by summer. One of the effects of heat shock on bovine oocytes is decreased nuclear maturation [26]. According to Maya-Soriano *et al.* [27], when bovine oocytes matured under heat shock circumstances, the oocytes collected in hot and cold seasons were similarly affected in terms of nuclear maturation. However, a seasonal influence was observed in cytoplasmic maturation. HS during summer was found to impair both nuclear and cytoplasmic maturation of bovine oocytes, reduce cortical granule translocation to the oolemma [28], and decrease cytoskeleton rearrangement [29], and spindle formation [30]. Such changes could result in insufficient nuclear maturation [28].

HSP70, a proapoptotic factor known to act during the unfavorable phase, was increased in both COCs and IVM oocytes [7]. The expression HSP70 rises in cumulus cells subjected to high temperatures in cattle, which may suggest a mechanism for heat stress in the seasonality of buffalo reproduction [31]. IVM of buffalo oocytes during the non-breeding season showed upregulation of b-actin (ACTB), a component of the actin cytoskeleton, involved in cortical granule translocation, and upregulation of glyceraldehyde-3-phosphate dehydrogenase, an enzyme responsible for energy metabolism [32]. This is consistent with the disruption of cortical granule translocation [28] and premature aging caused by accelerated cytoplasmic maturation reported in oocytes subjected to 41C during maturation in cattle [33].

Effect of Seasons on Embryo Developmental Competence in Buffalo

Results of this study indicated that there is a significant difference in the cleavage and blastocyst rates between the cold season (78.04, and 11.60 %, respectively) and the hot season (69.36, and 6.43 %, respectively).

These findings were consistent with those of Shahzad et al. [34], who found that the non-breeding season had poor blastocyst and hatching rate while the autumn season (Oct-Dec) had the highest efficacy of oocyte and embryo development parameters like oocyte maturation, blastocyst, and hatching rate. Abdoon et al. [7] displayed that cleavage rates were decreased ($P < 0.01$) during the hot season than during the cold season. Oocytes that matured in the cold season revealed a greater ($P < 0.01$) blastocyst rate than those that matured in the hot season. Zheng et al. [25] found that there are no significant changes in the fusion and cleavage rates throughout all seasons. The blastocyst rate was greater ($p < 0.05$) in autumn and winter than in the spring and summer seasons. However, Manjunatha et al. [35] postulated that there is no significant difference in hatching and blastocyst rates between breeding and non-breeding seasons.

The temperature increase has an impact on the developmental ability of the GV stage in bovine oocytes [36]. In comparison to the cool season, a lower proportion of these GV-stage oocytes after fertilization continue to the blastocyst stage in the warm season [37]. On the contrary, in vitro experiments [38] showed that higher temperature had no impact on the rate of oocyte cleavage

however, reduced the rate of blastocyst formation [39]. Hence, genetic variations in species' susceptibilities to high temperatures or variations in the length of exposure to or intensity of temperature may be the cause of these variations.

One of the mechanisms by which HS adversely affects embryonic development is hypothermia-induced oxidative stress. HS increases ROS in preimplantation bovine embryos (40) and reduces glutathione concentration in mouse embryos [41]. Heat-induced changes in the intrinsic quality of the oocyte could explain the reduction in early embryonic growth. Seasonal changes in maternal transcripts are one possibility. Early cleaved embryos with high developmental potential differ in their expression of GAPDH, GDF9, and POU5F1 transcripts, genes involved in early embryonic development, during the hot season. Likewise, during the summer, repeat-breeder cows had impaired expression of genes related to oocyte maturation (BMP15, GDF9, and FGF8, -10, -16, and -17) [42]. Another possibility is that HS damages cytoplasmic organelles in the oocyte, like the mitochondria, which are essential for early embryonic development. Additionally, heat stress caused apoptosis in bovine oocytes and a rise in phosphatidylserine, a marker of apoptosis, in swine oocytes [43,44].

Conclusion

Buffalo oocyte quality and yield are significantly affected by season. The cold season significantly increases the rate of in-vitro maturation and the transferable embryo rate (morula and Blastocyst) in buffalo.

Declarations

This study was funded by the Academy of scientific research and Technology, through the project Egypt-China Agreement 2022-2025.

Ethical Approval/Considerations:

This study was carried out according to standard protocols without causing discomfort or injury to the buffalo. Furthermore, the experimental procedure was approved by the Centre for Research and Community Service at National Research Centre, Dokki, Cairo, Egypt.

Ethical considerations (e.g., plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been made by the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors acknowledge the financial support through the Academy of scientific research and Technology of Egypt's agreement with the national natural science foundation of China through project (2022-2025) ID: 9154 titles (Heat Stress-induced Infertility in dairy cows: Molecular Basis of reduces oocyte quality and potential solution) and this work done in the Embryo and Genetic Resources Conservation Bank in the National Research Centre.

Authors' contribution

Omaima Kandil designed the experiment and supported all the equipment, chemicals, and facilities, Kholoud Alhallag brought the samples and did lab work, Esraa Ali Ismael and Omaima Kandil helped in lab work, Omaima Kandil, Said Fathalla, Sherif Shawky, Ibrahim Abu-Alya, Esraa Ali Ismael help in write the manuscript and analysis of data.

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تأثير الفصول على نشاط المبيض وكفاءة نمو البويضات والأجنة المنتجة معمليا في الجاموس

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أهداف الدراسة الحالية هي دراسة تأثيرات الفصول على (١) إنتاجية البويضات ومعدل الشفط وكفاءتها في الجاموس. (٢) معدل النضج والكفاءة نمو الأجنة المنتجة معمليا في الجاموس. المواد والطرق: تم تجميع مبيضات الجاموس خلال مواسم مختلفة من العام (٢٠٢١). الموسم البارد (من أكتوبر إلى مارس) والموسم الحار (من أبريل إلى سبتمبر) ، وتم حساب مؤشر درجة الحرارة والرطوبة (THI). تم جمع البويضات وسجل العدد الكلي للبويضات المتجمعة في كل موسم وتم حساب متوسط عدد البويضات بالنسبة لكل مبيض ومعدل التجميع في كل موسم. صنفت جودة البويضات الي ممتازة، جيدة، مقبولة، رديئة. تم إضاج البويضات الممتازة والجيدة معمليا في الوسط (TCM-199+ 10% FCS+50 u/ml gentamycin + 10ug FSH) عند درجة حرارة 38.5°م و5% ثاني اكسيد الكربون لمدة 22 ساعة. لتقييم نضج البويضات بعد نهاية فترة التحضين عن طريق تقييم النضج السيتوبلازم (الخلوي) بأربعة درجات من تمدد خلايا التراكم المبيضي (G0, GI, GII, GIII). ويحسب معدل نضج النواة عن طريق معرفة عدد البويضات التي تحتوي على الجسم القطبي الأول. تم إخصاب البويضات الناضجة ذات الجسم القطبي الأول باستخدام السائل المنوي المجمد، والتي تم تحضينها بعد ذلك لمدة ١٨ ساعة قبل زراعتها معمليا لمدة ٧ أيام بواسطة سائل قناة البيض الاصطناعي (SOF). أظهرت النتائج أن العدد الإجمالي والمتوسط لعدد البويضات لكل مبيض للجاموس في المواسم الباردة كان أعلى معنويا بشكل ملحوظ ($P < 0.05$) مقارنة بالمواسم الحارة. لا يتأثر معدل التجميع بشكل كبير بالموسم. تتميز المواسم الباردة بزيادة عدد ونسبة البويضات الممتازة والجيدة أيضا، كما تتميز المواسم الحارة بانخفاض معدل نضج بويضات الجاموس ($P > 0.05$). زيادة معدل الأجنة القابلة للنقل (البلاستوسيسيت blastocyst والموريولا morula) أعلى معنويا بشكل ملحوظ ($P < 0.05$) في المواسم الباردة مقارنة بالمواسم الحارة. الخلاصة، المواسم الحارة من فصول السنة لها تأثير سلبي على التكاثر في الجاموس عن طريق تقليل إنتاجية البويضات وقله كفاءتها. المواسم الباردة تتميز بزيادة معدل نضج البويضات ومعدل انتاج الأجنة القابلة للنقل في الجاموس.

الكلمات الدالة: فصول السنة ، نضج البويضات ، الاخصاب المعلمي ، الأجنة ، الجاموس.