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Seasonal variation in ovarian functions in Egyptian buffalo and cattle

Seham S. Soliman[1], Mahmoud Z. Attia, Ahmed S. Abdoon[1]*, Nahed El-Sayed El-Toukhey[2], Omaima M. Kandil[1] and Hussein A. Sabra[1]

[1]Department of Animal Reproduction and Artificial Insemination, Veterinary Research Division, National Research Centre, Egypt.
[2]Department of Animal Physiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

Abstract : In the bovine (cattle and buffalo) fertility is a multifactorial process and is affected by environmental, genetic, disease and management factors. In the bovine, summer season annually causes huge economic losses to the global dairy industry. The present work was designed to compare the reproductive patterns in buffalo and cattle under the same environmental conditions during the four seasons of the year. This include the effect of season of the year on: 1) reproductive status; 2) effect of season on oocytes quality and recovery rate; and 3) effect of season on oocyte maturation rate *in vitro* in buffalo and cattle. Our results showed significant differences in reproductive activity between the four seasons. In cattle and buffalo the highest ($P<0.01$) incidence of ovarian activity was recorded during winter and spring when compared with summer and autumn. Oocytes recovery rate was significantly higher ($P<0.01$) in winter and spring than in summer and autumn. The percentages of excellent and good quality oocyte were significantly higher ($P<0.01$) during winter and spring than summer and autumn, while, fair and denuded oocyte were significantly higher ($P<0.01$) in summer and autumn than winter and spring in buffalo and cattle. Consequently, oocyte maturation rate was higher ($P<0.01$) during winter and spring than summer and autumn. The results revealed that, in the cold environmental conditions the reproductive status, oocytes quality, yield and their maturation rate were higher in contrast to hot period in cattle and buffalo.
Key words: Season, Ovarian activity, Oocytes yield, Oocytes quality, maturation, buffaloes, cattle.

Introduction

Bovine (Cattle and buffaloes) are the most important farm animals worldwide**.** In the bovine, fertility is a multifactorial process and is affected by environmental, genetic, disease and management factors. In the cattle, heat stress during summer season annually causes huge losses to global dairy industry, including decrease in animal performance, metabolic disorder and health problem. Moreover, buffaloes are poor thermoregulatory animals due to their morphological and anatomical peculiarities. Environmental factors have a direct effect on the reproductive process at ovulation, fertilization, implantation or during gestation and parturition. In cattle, conception rates drop from about 40–60% in colder months to 10–20% or lower in summer[1]. Also, high ambient temperature during summer affects the duration and intensity of expression of estrus and increases the duration of anestrus and silent ovulation and is responsible for low pregnancy rates and

high embryonic losses in cattle[2]. The situation is more drastic for buffalo, inactive ovaries represent 93% of summer infertility in buffaloes raised in Middle Egypt[3]. The physiological mechanisms underlying the effect of summer season on fertility of dairy cows still remain elusive. Heat stress during summer season can compromise the ovarian follicles and their enclosing oocyte[4,5]. Bovine oocytes recovered during summer season exhibited lower quality and developmental rates[6,7]. Furthermore, assessment of the potential direct impacts of climate change on the reproduction of buffaloes indicate that there is increasing trend in incidences of silent estrus, the decline in reproductive activity and conception of buffaloes due to increase in air temperature during summer[8,9]. High environmental temperature had a detrimental effect on the yield, quality and developmental competence of buffalo oocytes[10]. [11]concluded that in summer months, the quality and number of oocytes decreased in buffaloes. Up to now, there is no available literature comparing the effect of season on ovarian function in buffalo and cattle. Therefore, it appears necessary to study the reproductive patterns in buffalo and cattle during different seasons of the year. So far, to our best knowledge, this is the first attempt to elucidate the effect of season of the year on the reproductive pattern in buffalo and cattle at the same time. This include the effect of season of the year on: 1) reproductive status; 2) effect of season on oocytes quality and recovery rate; and 3) effect of season on oocyte maturation rate in buffalo and cattle.

Materials and Methods

All chemicals and reagents used in the present study were purchased from Sigma-Aldrich (Sigma-Aldrich, Germany) unless otherwise mentioned. This work was approved by the Ethical Committee for Animals Research and Care, at the National Research Centre, Egypt.

Experiment 1: ***Effect of season on reproductive status and follicular development***

Genitalia form buffaloes and cattle were collected at El Monibe and El Waraque slaughterhouses during summer season (from June to the end of September 2011, n= 63 and 127 for cattle and buffalo, respectively) and winter season (from October 2011 to the end of March 2012, n= 47 and 186 for cattle and buffalo, respectively). Genitalia were classified according to the reproductive status (according to the macroscopic observation of the ovaries (presence, shape and size of follicle and CL) and the uterus (size, color, consistency, mucus) into cyclic, early pregnant or anestrous according to the methods adopted for cattle[12] and buffalo[13].

Experiment 2: ***Effect of season recovery rate and quality of cumulus-oocytes-complexes (COCs)***

Collection of ovaries

Pairs of cattle and buffalo ovaries were collected in plastic bags shortly after slaughtering of the animal, and transferred to a thermos containing 0.9% sterile normal saline solution. Ovaries were transported to the lab within two hours. Upon arrival to the lab, extra tissues were removed, then ovaries were washed once with 70% ethanol, followed by at least 3 times with warm saline sol. containing 100 IU/ml penicillin and 100 µg/ml streptomycin and keep in a beaker in water bath at 37ºC.

Oocytes recovery in cattle and buffalo

The ovaries were dehydrated with sterilized paper. Before oocytes gathering. Cumulus oocyte complexes (COCs) were obtained by aspiration method. follicles that had 2 – 8 mm in diameter were collected using a 10 ml sterile syringe and an 18 G disposable needle containing 1 ml phosphate buffer saline formed from 50 ml PBS + 4.0 mg/ml Bovine Serum Albumin (BSA) + 50 µg/ml gentamicin sulfate. Aspirated follicular fluid (bFF) containing the COCs put into 50 ml Falcon tubes in a water bath (38^{o}C) for about 15 minutes to stay in the depths of the tube. The bottom layer of this tube containing the COCs and other debris were collected using a sterile pipette and transferred to (90 mm) sterile Petri dish. The oocytes were chosen and washed three times with maturation medium (TCM -199) under a stereomicroscope.

Assessment of COCs quality and classification

The oocytes were counted and its number was recorded under stereomicroscope (28 x) and classified under stereomicroscope (56x) according to [14] into four groups depend on their morphology (cumulus cells and homogeneity of the cytoplasm) in this way:-

Excellent quality: Oocytes with four or more layers of complete cumulus-cells and evenly granulated dark cytoplasm.

Good quality: Oocytes with 1-3 layers of cumulus-cells and evenly granulated dark cytoplasm.

Fair quality: Oocytes with cumulus-cells incompletely surrounding the oocyte.

Denuded: Oocytes without cumulus cells and covered by zona pellucida.

The number and quality of COCs recovered were determined every season.

Experiment 3: ***Effect of season on in vitro maturation (IVM) of cattle and buffalo COCs.***

All chosen oocytes for IVM was washed three times with IVM medium, then oocytes were cultured in maturation medium which consisted of TCM- 199 supplemented with 1000 µg fetal calf serum + 10 µg/ml follicle stimulating hormone (FSH) + 10 IU human chorionic gonadotropin (hCG) +50 µg/ml gentamicin). Oocytes cultured in groups 25 oocytes/500 µl maturation medium in four-well culture dish . All media solutions were sterilized before use by passing through Millipore membrane filter (0.22 µm) fitted with 10 ml syringe to remove bacteria particulates. Oocytes were cultured at 38.5ºC, 5% CO_2 for 22 - 24 hours. The degree of cumulus cell expansion was determined after 22 - 24 h of IVM[14]:

- Grade zero (G0): with no expansion.
- Grade one (G1): with slight expansion in the outer layer of cumulus cells.
- Grade two (G2): with moderate expansion.
- Grade three (G3): with full expansion.

In addition, presence of first polar body was considered as a criteria for oocytes nuclear maturation.

Statistical analysis

All Data were expressed as mean ± standard error (S.E.).The significance of differences was tested by paired *t*-test and analysis of variance (ANOVA) followed by post hoc test. Means were compared by the least significance difference at *P<0.05 and **P<0.01. All tests were performed using computer package of the Statistical Analysis system (SPSS, version 16, 2007).

Results:

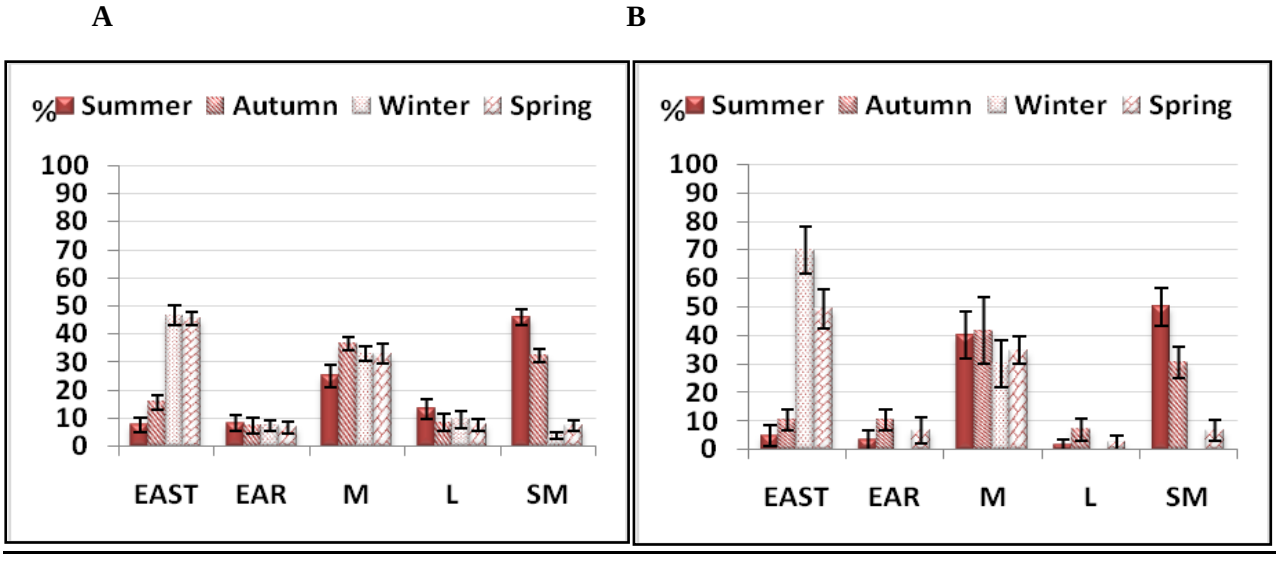

Fig. 1: Histogram showing the reproductive status in buffalo (A) and cattle (B) during the different seasons of the year.

Result presented in Figure 1 A,B indicated that after the morphological examination of genital tracts for buffalo and cattle there was a seasonal variation in ovarian activity. Both buffalo and cattle showed higher percentage (P<0.01) of smooth inactive ovaries during summer and autumn seasons compared to winter and spring seasons, and showed a higher (P < 0.01) percentage of estrus stage during winter and spring compared to summer and autumn, However, no significant difference was detected in early luteal, mid luteal and late luteal stages between different seasons. Overall, the percentage of animals in estrus was higher (P<0.01) in cattle than buffalo, and there was no significant difference in other reproductive stage between buffalo and cattle (Fig. 1A,B).

Effect of season on number of ovarian follicles in cattle and buffalo

The effect of season on the total number of ovarian follicles in buffalo and cattle is demonstrated in Figure 2. Results indicated that the total number of ovarian follicles was significantly higher (P<0.01) in both buffalo and cattle during winter season followed by spring when compared with summer and autumn seasons. Overall, number of ovarian follicles was significantly higher (P<0.01) in cattle than buffalo (Fig. 2)

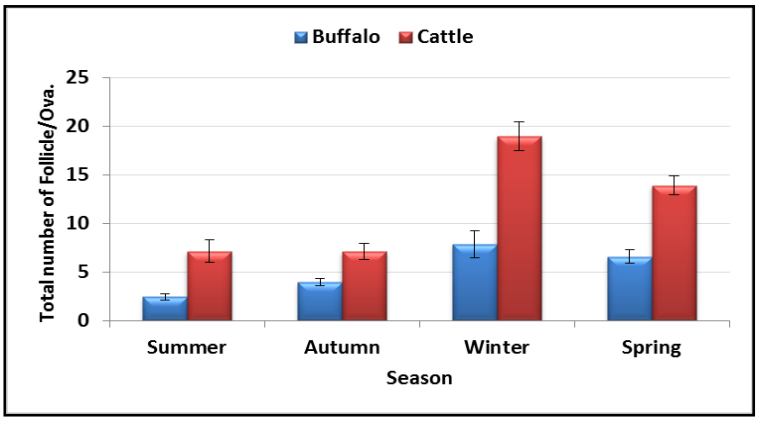

Fig. 2: Histogram representing the effect of seasons on the number of follicles in cattle and buffalo ovaries.

Effect of season on oocytes number in cattle and buffalo:-

Data presented in Figure 3, revealed that the number COCs recovered was significantly differ between the four seasons (P<0.05). In buffalo oocyte recovery rate increased significantly in winter and spring, on the other hand it was significantly (P<0.01) lower in autumn and summer. As well as in cattle, it increased significantly in spring and winter but it decreased significantly in summer and autumn, so recovery rate of oocyte from ovary is was significantly higher (P<0.001) in cold seasons than hot seasons in buffalo and cattle. Overall, oocyte recovery rate was significantly higher (P<0.01) in cattle than buffalo (Fig. 3).

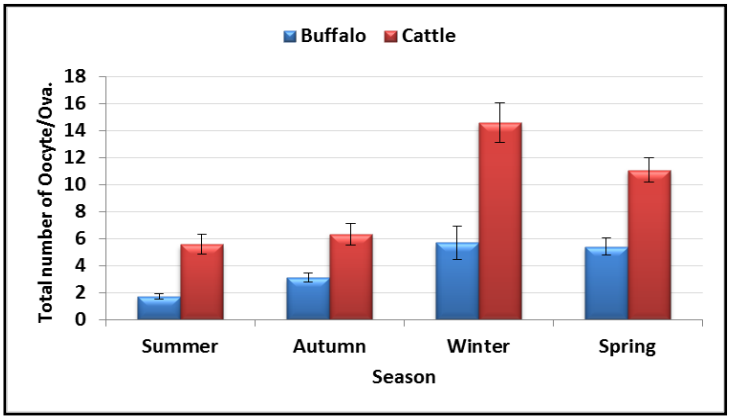

Fig. 3: Effect of season on recovery rate of cattle and buffalo COCs.

Effect of season on oocyte quality in buffalo and cattle

Result summarized in Figure 4 demonstrated significant difference in COCs quality between the four seasons (P<0.01) for buffalo (Fig. 4A) and cattle (Fig. 4B). In cattle and buffalo, the percentages of excellent and good quality COCs recovered during winter and spring were significantly higher (P<0.01) than that recovered during summer and autumn. Meanwhile, in cattle there was no significant difference in the percentages of fair and denuded oocytes during the four season (Fig. 4A). Moreover, in cattle the percentage of fair quality oocytes were significantly lower (P<0.01) during winter than in other seasons. While, there was no significant difference in the percentage of fair and denuded oocytes between summer, autumn and spring in cattle (Fig. 4B). During winter and spring the percentage of excellent quality oocytes was higher in buffalo than cattle.

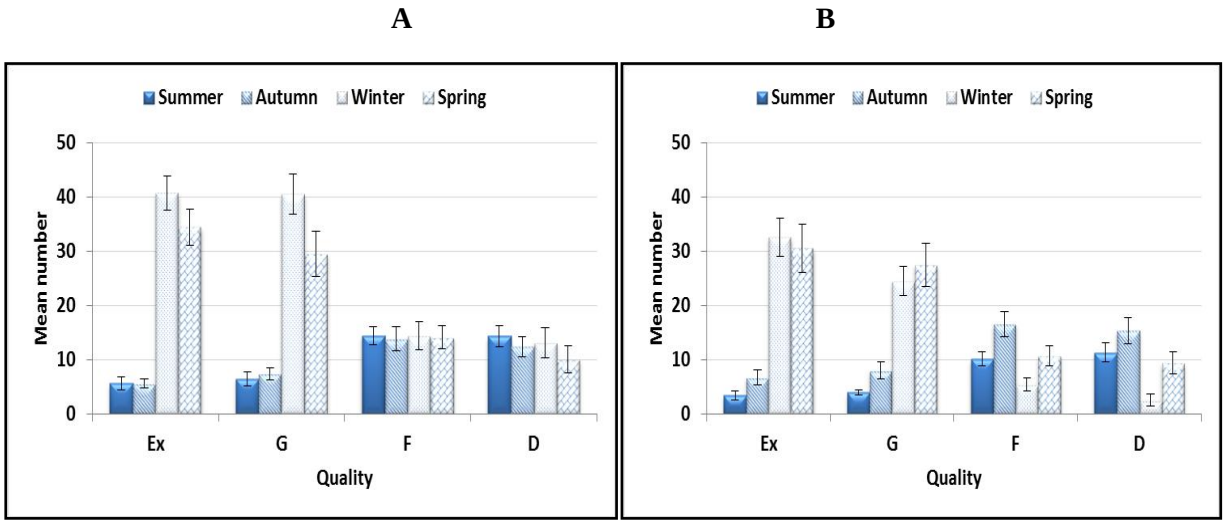

Fig. 4: Histogram showing difference in COCs quality during different seasons in buffalo (A) and cattle (B).

Effect of season on maturation rate of buffalo and cattle oocytes

Figure 5 showed the degree of cumulus-cell expansion in buffalo and cattle oocytes during the different seasons of the year. In both cattle and buffalo, the highest (P<0.01) percentage of Grade 3 and Grade 2 cumulus-cell expansion of in vitro maturated oocytes was recorded during winter and spring seasons compared with summer and autumn, while, the highest (P<0.01) percentage of Grade 0 and Grade 1 cumulus-cell expansion was reported during summer and autumn seasons.

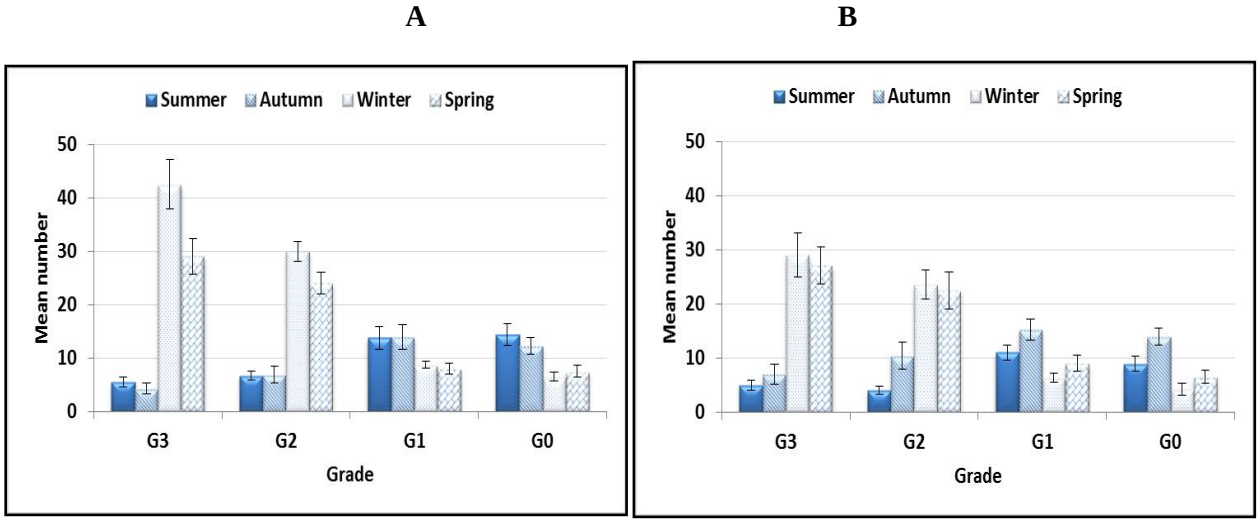

Fig. 5: Histogram showing the percentages of different Grads of cumulus-cell expansion of in vitro matured buffalo (A) and cattle oocytes (B) during the different seasons of the year.

It is clear from Figure 6 that buffalo and cattle oocytes showed significantly higher (P<0.01) nuclear maturation rate in winter and spring when compared with their maturation rate during summer and autumn. Oocyte maturation rate was very closed between buffalo and cattle throughout the different seasons (Fig. 6).

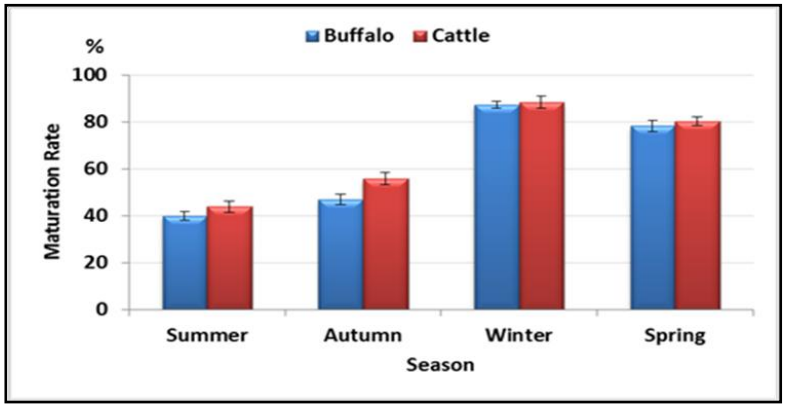

Fig. 6: Effect of season on maturation rate of oocyte in buffalo and cattle.

Discussion

Effect of season on bovine ovarian functions is a factor of critical impact. High environmental temperature during hot seasons annually causes huge economic losses to dairy industry worldwide, including decrease in the productive and reproductive performance as well as disease resistance. The decreased fertility associated with summer heat stress is a multifactorial problem in which hyperthermia affects cellular function in various tissues of the female reproductive tract[15,16]. In the present work, results showed that there were significant differences between the four seasons in ovarian activity of cattle and buffalo, and these was reflected on the reproductive activity, the quality and the yield of oocytes and their subsequent maturation rate. In cattle and buffalo, the reproductive status is greatly affected by season, the highest incidence of animals in estrus was recorded during winter season, while, the highest incidence of smooth inactive ovaries was recorded in summer season. Meanwhile, incidence of animals in early luteal, mid luteal and late luteal phase did not vary between different seasons. A significant change in conception rate of cattle and buffaloes was observed in response to the high temperature and humidity. Ovarian inactivity is the main cause of infertility in cattle and buffaloes in Middle Egypt, and there is a link between poor nutrition in summer and the high incidence of ovarian inactivity [3]. These results are completely agree with that previously recorded in Egypt for cattle[17,18] and buffaloes[10]**.** In cattle, high ambient temperature affects the duration and intensity of expression of estrus, and increases the duration of anestrus and silent ovulation[2]. Lactating dairy cattle are sensitive to heat stress because of high metabolic heat production inside the body, which associated with increased milk production. Also, the percentage of cows in estrus was higher (P<0.05) in cattle during winter and spring compared with buffaloes. Expression pattern of heat shock protein genes indicated that the expression was higher in buffalo compared to cattle, indicating that cattle are more tolerant to heat stress than buffaloes [19].

Moreover, in the current work, the number of follicles and the number of oocytes recovered per ovary were affected by season in buffalo and cattle. The highest number of follicles and the highest number of oocytes recovered per ovary was recorded during winter and spring when compared with summer and autumn. Also, oocytes recovery rate was higher in cattle than buffalo during all seasons. Similarly, [20] concluded that there was a significant decrease in the buffalo oocyte recovery rate from the slaughterhouse ovaries in hot season than that in spring and winter (cold season). The poorer yields of functional oocytes in the hot season may due to high temperature that changed endocrine patterns and reduced the development of follicles[21], or hot seasons may decrease ovary sensitivity to gonadotropin stimulation[22]**,** and disrupt steroidogenesis[5]**.**

Furthermore, oocyte is a central regulator of multiple aspects of female fertility, including ovarian follicular development and early embryogenesis. In the present work season has direct effect on oocytes quality, the highest percentage of excellent and good quality oocytes in buffalo and cattle was recorded during winter and spring, and the highest percentage of poor quality and denuded oocytes was recorded in summer season for both buffalo and cattle. These results are concomitant with that previously recorded in buffalo [10,21,23] and cattle [6] High temperature has a direct effect on follicle maturation [24]. High environmental temperature during summer

can compromise the oocyte and the follicle in which it is encased, and alters bovine follicular population and oocytes quality [5]. Bovine oocytes are more susceptible to high temperature during the preovulatory period [25]**.** In addition, Bovine oocytes recovered during summer season exhibited lower quality and developmental rates [6,26,27]**.** [28]recorded that high environmental temperature inhibits ovarian follicular development leading to diminished reproductive efficiency of dairy cows during summer, and high temperature induced granulose cell apoptosis through the BAX/BCL-2 pathway and reduced the steroidogenic gene messenger RNA (mRNA) expression and E2 synthesis. [29] concluded that thermal seasonal stress can alter phospholipids composition of oocyte so affect on its quality. Interestingly, in the current work, the percentage of excellent and good quality COCs was higher in buffalo than cattle, this could be due to the effect of age of the slaughtered animals, as most of the cows slaughtered are senile.

Although, in this work, season adversely affect oocytes maturation rate in buffalo and cattle. A significantly high ($P<0.01$) oocyte maturation rate was achieved during winter and spring than during summer and autumn for both buffalo and cattle. Similarly, maturation rate was significantly higher for buffalo oocytes recovered in cold season than in hot season [10,21] and bovine oocytes[6,7]. Thermal stress impairs intracellular actions related to both nuclear and cytoplasmic maturation. For example, translocation of cortical granules to the oolemma[30], cytoskeletal rearrangement and spindle formation[31]. [10] reported that the low maturation rate of buffalo oocytes during hot season was attributed to the over mRNA gene expression of heat shock protein 70.

In conclusion: Summer and autumn seasons impair reproductive performance in cattle and buffalo under Egyptian conditions by increase the incidence of smooth inactive ovaries, and decrease oocytes quality, quantity and their maturation rate in vitro. A proper management system like air fane placing animals in shadow places during summer season could alleviate the drastic effect of high temperature on reproduction in Egyptian buffaloes and cattle.

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