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Season results on the norm and thus in vitro development estimation of *Bubalus bubalis*

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Abstract

Recent advances in *in vitro* maturation, fertilization and culture technology allowed progress in an increasing the number of off springs produced from genetically superior females but this progress still has important factors affect the yielding and quality of the oocytes. It was referred that, there is a distinct similarity in season profile between an *in vitro* and *in vivo* reproductive traits. The influence of season on oocyte quality has been largely considered, as a factor of high impact. The purpose of this paper was to study the effects of season on the quality of oocytes and its relationship with cumulus cells expansion, maturation and developmental rates of *in vitro* maturation of Egyptian buffalo oocytes. The main goal of our investigation was shown to evaluate the role of change in the temperature during the year on the buffalo and consequently affecting the quality of their oocytes. The results showed significant differences between the four seasons. In spring and winter, the percentage of good quality oocytes was (71 and 74.6%), denuded oocytes was (12.8 and 9.1%) and the fair type of oocytes was (16.3 and 16.3%) respectively. Consequently the maturation rate was 85.5 and 92.5% respectively. While in summer and autumn, the percentage of good quality oocytes was (50 and 56.9%) instead of denuded oocytes was (25.5 and 18.5%) and the fair type of oocytes was (24.4 and 21.5%) respectively. Consequently the maturation rate was 59.6 and 74.5% respectively. The results revealed that, when the percentage of good oocytes increased, cumulus expansion and the maturation rate had increased. The higher degeneration of oocytes in the hot period reflects their lower developmental competence of oocytes compared with recovered in the cool environmental conditions.

Keywords: Oocytes, season, maturation, buffaloes

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INTRODUCTION

Research in technological manipulation of the mammalian oocytes has progressed very rapidly over the last decade. The transfer of *in vitro* embryos from slaughter house buffalo ovaries provides an opportunity to increase the reproductive efficiency. Buffaloes are seasonally polyestrous and are reproductively less active in summer.

In buffalo, the incubation temperatures during the *in vitro* maturation (IVM) influenced the fertilization rate but had no significant effect on maturation and subsequent embryo development (Ravindranatha et al., 2003).
The incubation temperature of 38.5ºC during IVM was found to be optimum for embryo production *in vitro*.

Al-Katanani et al. (2002) concluded that summer depression in oocyte quality in Holstein cows was evident, but cooling cows for 42 days did not alleviate that seasonal effect.

Singla et al. (1999) reported that buffaloes subjected to heat stress yield fewer good quality oocytes than their unstressed counterparts the developmental competence of the oocytes under *in vitro* conditions has been investigated.

It is reported that the hypothalamo pituitary axis in sheep is quiscent under non stimulatory photoperiods which brought about by inhibitory influence of the penile

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gland while stimulatory photoperiods allows secretion of functional plateau of the gonadotrophins, (Karsch et al., 1989). Rutledge et al. (1999) emphasized that the production of cattle blastocyst was reduced in mid to late summer. This was preceded by increased viability from mid to late spring. Winter and fall months were characterized by stable, high yield of blastocysts. They also reported that, seasonal changes in cattle fertility may be caused by factors related to storage of the oocyte's developmental program. In contrast, Rocha et al. (1997) in Bos indicus (Brahman) cows and Rivera et al. (2000) in bovine failed to indicate an effect of season on *in vitro* production of embryos in a subtropical environment.

Seasonality is less pronounced between males of domestic species, but there are still differences in behavior and sperm characteristics depending on the time of the year (Gerlach et al., 2000, Ciereszko et al., 2000 and Chacon et al., 2002).

Our study was to investigate the effects of the seasons on the quality of Egyptian buffalo oocytes and consequently their *in vitro* maturation rate.

MATERIALS AND METHODS

Oocyte collection

The ovaries were separated shortly after slaughtered Egyptian female's buffalo and maintained in a thermos flask containing 0.9% sterile normal saline and gentamycine 0.5 mg/ml. Ovaries were transported to laboratory within two to three hours (Leibfried et al., 1987).

Under aseptic condition the ovaries were washed with Ethanol 70% (to remove adhering blood), and avoid contamination. Other tissues were dissected away from the collected ovaries, then the ovaries were rinsed by sterile wormed saline at 35 - 37ºC (Totey et al. 1991a) and Totey et al. 1993b) three times to further remove any contaminants on the ovary surface and the traces of ethanol.

Recovery of immature oocytes

Before oocytes collection, the ovaries were dried with sterile paper. Cumulus oocyte complexes (COCs) were obtained by aspiration of follicles that had 2 – 8 mm in diameter using a 10 ml sterile syringe and an 18 G disposable needle containing 1 ml PBS (8.09 gm/l NaCl, 0.2 gm/l, 1.15 gm/l and 0.29 gm/l) with 3 mg/ml BSA. The aspirated buffalo follicular fluid (bFF) containing the COCs were placed into 50 ml Falcon tubes in a water bath (38oC) for about 15 min to settle in the bottom of the tube. The bottom layer of bFF containing the COCs and other follicular debris were recovered using a 5 ml sterile pipette and transferred to sterile Petri dish (90 mm). The collected oocytes were selected and washed three times with maturation medium under a binocular microscope.

Oocytes classification

The oocytes were counted and classified into three classes based on the cumulus cells and homogeneity of the cytoplasm as recommended by Leibfried and First (1979), Shioya et al. (1988) and modified by Ganguli et al. (1998).

i. Class 1 (CCOCs): Oocytes were completely invested with cumulus cell layers (Good oocytes).
ii. Class 2 (SCOCs): Oocytes were surrounded with scantly cumulus cell layers (Fair oocytes)
iii. Class 3 (Nude): Naked (denuded) oocytes.

IVM technique

All selected oocytes for IVM was washed three times with IVM medium, then oocytes were cultured in maturation medium which consisted of TCM- 199 (25 mM HEPES, with Earleis salt and L-glutamine, Sigma USA) supplemented with 10% buFF, 5 IU/ml eCG (Folligon, Inver, Netherland) and 5 IU/ml estradiol hormone and covered with mineral oil (Sigma, USA) (oocytes cultured in groups 25 oocytes/droplet) . All media solutions were sterilized before use by passing through Millipore membrane filter (0.22 µm) in diameter fitted with 10 ml syringe to remove bacteria particulates. The medium was freshly made before the work by two hours, from stock solution of each compound (Leibfreied and First, 1979). Oocytes were cultured at 38.5ºC, 5% CO2 and 95% humidity for 22 - 24 h. All solutions in this work contained antibiotics 10 µg/ml gentamycin (Aoyagi et al., 1990).

The degree of cumulus cell expansion was determined after 22 - 24 h of IVM as at Degree-0 (no expansion) and Degree- 1 (cumulus cells were homogeneously spread). Also presence of first polar body was the criteria for maturation of the oocyte.

Statistical analysis

The obtained data were analyzed by ANOVA one way analysis. It was analyzed using the analysis of variance (ANOVA) procedure using M state Program.

RESULTS

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

Table 1 showed significant differences between the four seasons ($P < 0.05$). In spring months (Month 3, 4 and 5 of the year) and winter months (12, 1 and 2 of the year) the percentage of good quality oocytes was 71 and 74.6%, denuded oocytes was 12.8 and 9.1%, and the fair type of oocytes was 16.3 and 16.3% respectively, and consequently the maturation rate was 85.5 and 92.5%.

While in the summer months (6, 7 and 8 of year) and autumn months (9, 10 and 11 of year) the percentage of good quality oocytes was 50 and 56.9%, denuded oocytes was 25.5 and 18.5%, and the fair type of oocytes was 24.4 and 21.5% respectively, and consequently the maturation rate was 59.6 and 74.5% at ($P < 0.05$) respectively.

The quality of buffalo oocytes and its relation to the maturation rate

The quality of buffalo oocytes was studied through out study and we evaluated the relationship between the quality of oocytes (Good, Fair or denuded) and the *in-vitro* maturation of buffalo oocytes Table 2. The results revealed that when the percentage of good oocytes was increased and denuded oocytes decreased, the matura-

Table 1. The relationship between the seasons and the quality of the oocytes and their maturation rate in Egyptian buffalo.

Month (Season)	No. of Ovaries in Mean ± S.E.	No. of Oocytes In Mean ± S.E.	No. of oocytes/ ovary (yield)	Oocyte Quality						Maturation rate	
				Good		Fair		denuded			
				No. of oocyte in Mean ± S.E.	%	No. of oocyte in Mean ± S.E.	%	No. of oocyte in Mean ± S.E.	%	No. of oocyte in Mean ± S.E.	%
3, 4 and 5	120	246.7	2.1	175.0	71^{b}	40.0	16.3^{a}	31.7	12.8	215	85.
(Spring)	± 2.4	±3.2		± 5.7		± 2.7		±3.2	^{a}	± 5.0	5^{c}
6, 7 and 8	102	176.7	1.7	88.3 ±1.7	50 ^{a}	43.3	24.4^{b}	45	25.5	106.6	59.
(Summer)	± 0.3	±1.9				± 2.3		± 2.7	^{c}	±2.95	6^{a}
9, 10 and 11	130	246.0	1.9	140.0	56.9^{a}	53.3	21.5^{b}	53.3	18.5	186.6	74.
(Autumn)	± 2.7	± 4.8		± 5.5		± 2.1		±1.6	^{b}	±5.2	5 ^{b}
12, 1 and 2	125	276.7	2.2	206.6 ±	74.6^{a}	45 ± 2.2	16.3^{a}	25	9.1 ^{a}	256.6	92.
(Winter)	± 1.2	± 4.8		0.4				± 2.6		±1.2	9 ^{d}

S.E. Standard Error Symbol a, b and c refer to the differences between the groups significant differences P < 0.05 and P > 0.01.

Table 2. The oocyte quality and its relationship to cumulus expansion and maturation rate in buffalo.

Trial	No. of Ovaries	No. of Oocytes	Oocyte Quality						Cumulus Expansion		Maturation Rate	
			Good		Fair		denuded					
			No. of oocyte	%	No. of oocyte	%	No. of oocyte	%	No. of oocyte	%	No. of oocyte	%
1	52	105	46	43.8	28	29.4	31	33.5	33	34.7	30	31.5
2	45	94	53	49.8	20	18.8	21	19.7	75	75.5	75	75.5
3	49	109	88	80.7	8	7.3	13	11.9	108	99	103	94.4
4	51	98	76	74.5	9	9.2	13	12.7	98	100	96	98

tion rate was increased as shown in Table 2.

DISCUSSION

Seasonality deeply affects the physiology and behavior of many species, and must be taken into account when biological resource banks (BRBs) are established.

Biological resource banks (BRBs) are important tools for the conservation of species and valuable breeds, and have been strongly developed during the last decade (Felipe Martinez-Pastor et al., 2005). They concluded that, the term BRB comprises many techniques and protocols, the purpose of which is to collect, preserve and utilize tissues and germplasm of selected individuals in order to ensure the continuity and the genetic variability of breeds, populations and species.

The influence of season on oocyte quality has been largely considered, as a factor of high impact. We have studied the effect of seasonality on the quality of oocytes and consequently their maturation rate in buffaloes. Our results showed that there are significant differences between the four seasons that consequently affected the quality and the yield of oocytes. Johnston et al. (1995) reported that, one simple method to achieve the objectives of BRB is the collection of sperm from males, followed by its cryopreservation and its use in artificial insemination (AI) when needed. This way, the genetic variability of a population can be maintained in an easy and inexpensive manner. So the effect of season in the collection of oocytes plays a critical role on the oocyte quality and its use in AI when needed.

Buffaloes are the main rural Egyptian economy, contributing milk, meat and draught power for the agriculture sector. However, delayed maturity; seasonality of breeding; poor conception rate; silent estrus; fewer follicles; higher rates of follicular atresia; high embryonic mortality; and poor superovulatory response are major construction to their reproductive performance and productivity.

Photoperiod, mediated through the hormone melatonin, is the main factor triggering events related with season (Webster et al., 1991; Lincoln 1998). There is abundant literature in this respect, and numerous studies have been carried out on circannual variations of many hormones and its importance on body, gonadal and gametogenesis changes (Goeritz et al., 2003; Gerlach and Aurich, 2000; Hoffmann and Landeck, 1999).

Pugh et al. (1991) claimed that the production of sheep embryos via *in vitro* maturation and fertilization techni-

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ques is possible in the non breeding season. The same finding was also obtained by Seydou et al. (1999) in goat. Moreover, Ingraham et al. (1974) observed that the exposure to high temperature and humidity 2 days before breeding were more detrimental to conception rates of dairy cows, than exposure of 1 day before or/ and 2 day after breeding.

In addition, Leibfried- Rutledge et al. (1989) reported that high ambient temperature and humidity have deleterious effects on oocyte capability for maturation and fertilization *in vitro*. While, Rehman et al. (1994) demonstrated that embryo viability of superovulated Holstein cows decreased from 59% on day 7 to 27% on day 14 in the hot season but did not change during the cool season.

On the other hand, Shi et al. (1991) 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. In the mare, Bruck et al. (1996) reported that the total oocyte recovery rate was significantly higher in May/June (57.3%) than in August/September (44.0%), there was no difference in the rate of metaphase II maturation after 36 h of culture in relation to the cyclic stage or season.

Al-Katanani et al. (2002) concluded that summer depression in oocyte quality in Holstein cows was evident, but cooling cows for 42 days did not alleviate that seasonal effect. Their results indicated that oocyte competence in Holstein cattle located in a warm climate decline during summer. Although cleavage rate was not reduced during warm weather, zygotes formed during the summer had reduced competence to develop to the blastocyst stage.

Zeron et al. (2001) concluded that there is evidence that heat stress can alter phospholipids composition of oocytes. Nandi et al. (2001) reported that external environmental conditions did not affect the fertilization if the aspirated oocytes successfully completed maturation. They examined the heat stress on the recovery, quality and developmental competence *in vitro* of buffalo oocytes. They showed that, the poorer yields of usable oocytes in the hot season may have been due to heat stress which altered endocrine patterns and reduced follicular development.

The efficiency of *in vitro* production (IVP) in buffalo is much lower than that in cattle and several problems need to be resolved before IVP technology in buffalo breeding, Nandi et al. (2002) . Neglia et al. (2003) reported that, in buffalo species the source of oocytes significantly affected post fertilization embryo development, as demonstrated by the higher blastocyst yields derived from OPU-derived oocytes.

A higher overall *in vitro* embryo production (IVEP) efficiency, mainly related to the higher cleavage rate, was recorded in cattle compared with buffalo when ovaries from an abattoir were used as oocyte donors.

In conclusion, our aim was to assess the differences in oocyte quality in different periods of the year, hence providing data that may be of use in the creation of germplasm banks for these and similar species. More in-depth research needs to be carried out on the quality of oocyte quality at different times of the year in order to confirm these findings.

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