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Effect of Reproductive Status on Yield and in Vitro Maturation of Oocytes of Egyptian Sheep

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ABSTRACT

The current study investigated the effect of breeding season and presence of corpora lutea (CLs) on the ovaries with, ovarian characteristics, and yield, quality and oocyte recovery rate in sheep. Also, the effect of addition of sheep serum (SS) and bovine serum albumin (BSA) to maturation medium of sheep oocytes during breeding and non-breeding season was studied. Ovaries were collected during breeding and non-breeding seasons from slaughterhouses. Ovaries were classified with or without CLs during both seasons. Oocytes were collected by slicing and their yield, category and recovery rate were determined. Only compact oocytes (COCs) were in vitro matured as affected by breeding vs. non-breeding season and addition of 10% SS vs. BSA. Results show that only ovarian weight was higher ($P<0.05$) in breeding than in non-breeding season and with CL-ovaries than without CLs. Ovaries were longer and thicker in breed in than in non-breeding season. CL-ovaries during breeding season showed the highest ovarian characteristics. Number of all visible follicles/ovary tended to be higher ($P<0.05$) in breeding than in non-breeding season and of ovaries with than without CLs ($P\geq0.05$). Oocyte yield/ovary was greater ($P<0.05$) by 41.7% in breeding than in non-breeding season. Oocyte yield was insignificantly greater on ovaries with CLs than those without CLs. Oocyte recovery rate was insignificantly higher in breeding and on ovaries with CLs than in non-breeding season and on those without CLs. Number/ovary and percentage of COCs were higher, while number/ovary were lower in breeding than in non-breeding season ($P<0.05$). Number and percentage of all categories were higher for oocytes recovered from ovaries with than without CLs. Percentage of oocytes at M-I stage was slightly higher in non-breeding than in breeding season. Percentage of oocytes at M-II showed an opposite trend, reflecting insignificantly higher maturation rate in breeding than in non-breeding season. Percentage of oocytes at M-I and M-II stages was insignificantly higher in BSA than in SS-medium. Oocyte maturation rate with SS than with BSA in breeding and non-breeding season. In conclusion, sheep oocytes were available to be harvested during non-breeding season from slaughtered ewes with acceptable yield, quality and the maturation rate in vitro by addition sheep serum (10%) to maturation medium.

INTRODUCTION

Development of biotechnology in sheep, ovine embryo production *in vitro*, is important for genetic improvement (Brackett *et al*., 1982), in which in vivo maturation (IVM) and fertilization (IVF) of bovine oocytes were used (Sirard, 1989). *In vitro* embryo production includes several processes (IVM, IVF and embryo culture). The oocytes IVM provides good opportunity for cheap and abundant embryos for application of basic research and emergency of biotechnology, such as transgenic and cloning (Li *et al*., 2006).

In Egypt, sheep are considered as meat, milk and wool sources. Technology of *in vitro* embryo production (IVEP) is a useful tool for improving genetic factors and increasing production efficiency (Gilchrist and Thompson, 2007). Sheep are considered short-day breeders and become sexually active by decreasing day length in late summer to early autumn (Rosa and Bryant, 2003). Sheep breeds in temperate climate zone (≥35°) had seasonality. Ewes are considered as completely seasonal or intermittently poly-estrus animals in regions between 35° North and 35° South. In these regions, breeding season occurred by declining photo period length. In Mediterranean zone, most of sheep breeds are comparatively unseasonal (Rosa and Bryant, 2003). Seasonality in reproduction of sheep was characterized by changes in sexual behavior, endocrine hormones and ovulation (Rosa and Bryant, 2003).

Effect of season on IVEP was studied by several authors on different animal species (Colleoni *et al*., 2004; Silva *et al*., 2006). Some authors failed to demonstrate an effect of season on IVEP in cattle in a subtropical climate (Rivera *et al*., 2000). However, different blastocyst yields in the hot as compared to cold seasons were reported in seasonally polyestrous Egyptian buffalo (Khairy *et al*., 2007). In cat, as a seasonal species like, the nuclear maturation of the recovered oocytes in non-breeding season is about 20%, but no embryos develop to the blastocyst stage (Spindler and Wildt, 1999).

Season has significant role on ovine reproduction, in term of some deficiencies in non-breeding seasons for IVM-IVF systems. So, the IVEP may be improved by the IVM system optimization (Carolan *et al*., 1994). Number of recovered oocytes/ewe, the oocyte developmental competence, and the IVF could be affected by seasonal anoestrous (Stenbak *et al*., 2001; Vázquez *et al*., 2009). Characteristics of the ovary as affected by season and nutrition have a marked effect on grading and recovery rate of oocytes (Ramsingh *et al*., 2013).

The season effect can be modulated by improving some environmental conditions of maturation medium in sheep. The basic media in most oocyte IVM experiments on animals is supplemented with various types of sera (Motlagh *et al*., 2008). Serum addition to the culture medium is considered as albumin source to give the osmolarity balance and to act as scavenger of free radicals (Thompson, 2000). Also, different types of sera had a wide variation in their contents of proteins (globulin and futuin), hormones and trace nutrients (Hsu *et al*., 1987).

Therefore, the present study aimed to investigate the effect of breeding season and presence of corpora lutea (CLs) on the ovaries with, ovarian characteristics, and yield, quality and rate of oocytes recovery of sheep. Also, the effect of addition of sheep serum (SS) and bovine serum albumin (BSA) to maturation medium of sheep oocytes during breeding and non-breeding season was studied

MATERIALS AND METHODS

This study was conducted at IVF laboratory, International Livestock Management Training Center, Sakha, Kafrelshiekh Governorate, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, in cooperation with Tanta University,

Faculty of Agriculture, Animal Production Department, during the period from January 2016 to September 2017.

Ovaries collection:

Ovaries were weekly collected during September-December (breeding season) and during March-July (non-breeding season) from private slaughterhouses in Borg El-Arab, Alexandria Province (about 225 km from the laboratory). Immediately after slaughtering animals, ovaries were isolated, placed in punctured plastic bag, then into thermos (28°C) containing 0.9% NaCl saline solution supplemented with 100 IU penicillin and 100µg streptomycin/ml. Thereafter, thermos with ovaries was taken within 3 hours to the laboratory.

Ovaries assessment:

In the laboratory, all excess tissues from the stalk of the collected ovaries were isolated. To clean adhering clotted blood, ovaries were washed two times by PBS (phosphate buffer solution warmed 28oC) supplemented with penicillin and streptomycin/ml (100 IU and 100 µg, respectively). Then, for removing any contamination on the ovarian surfaces, ovaries were washed with 70% ethanol one time.

Ovaries of breeding or non-breeding seasons were classified into ovaries bearing corpus luteum (CL) and ovaries without CL, then weighed and measured for its length, thickness and width using caliper.

Preparation of harvesting medium:

Phosphate The PBS (Sigma-Aldrich Chemie GmbH, P4417) as a harvesting medium was prepared by dissolving one tablet of PBS in sterile distilled water (200 ml) supplemented with 100 IU penicillin and 100 µg streptomycin/ml, and 2 mg/ml bovine serum albumin (BSA). Harvesting medium was adjusted to 7.2-7.4 pH (pH-meter) and 280-300 mOsmol/kg (osmometer), and filtered (0.22 µm-millipore) by filter (Milieux GV, Millipore, Cooperation Bedford, and MOA).

Oocyte collection and evaluation:

Pre-oocyte collection, count of ovarian follicles (≥2 mm) per ovary was determined, then oocytes were collected by slicing technique. Yield of oocyte, in term of number of oocyte/ovary was recorded. Oocyte recovery rate was calculated as the following:

Recovery rate = {recovered oocyte (n)/ follicles (n)} x 100

The collection oocytes were washed 3 times in the harvesting medium, and then each oocyte were evaluated and classified into four categories according to Madison *et al*. (1992) by stereomicroscopy into compact with ≥5 cumulus cell layers, partial denuded with incomplete cumulus cell layers surrounding the oocyte, denuded without any cumulus cell layers and surrounding by zona pellucida, and Shrunken or degenerated with shrunken or incomplete ooplasm away from the zona pellucida or with empty zona pellucida.

***In vitro* maturation:**

The TCM-199 medium containing 10% sheep serum or 6 mg/ml bovine serum albumin (BSA, 160096, Mp) was used as in vitro maturation medium to evaluate the impact of sera supplementation on IVM of sheep oocytes recovered during breeding season and non-breeding one. Sheep serum (SS) was prepared by collection of blood samples from adult ewes with unknown reproductive history, then serum was collected and stored at -20oC. Composition of TCM-199 is shown in the following table:

Content	Amount/ 10 ml	Production Co.
TCM-199	9 ml	Egyptian Orgnaization for Biological Product and vaccine, Agoza.
PMSG	20 µg/ml	Sigma Chemi. Co.
hCG	10 IU/ml	Epifasi, Egyptian Int. Pharmaceutical Industries Co, Egypt.
E-17β	1 µg/ml	Sigma Chemi. Co.
Na Pyruvate	20 mMol	Sigma Chemi. Co.
Na Penicillin G	100 IU/ml	Misr Co. for Pharm. Egypt
Streptomycin	100 µg/ml	Sigma Chemi. Co.

The medium The prepared maturation medium was adjusted to pH (7.2-7.4) and osmolarity level (280-300 mOsmol/kg) and filtered by 0.22 µm-millipore filter (milieux GV, millepore, Cooperation Bedford MOA).

Oocytes were washed three times in PBS with 2% BSA and 2 times in maturation medium to remove follicular fluid substances, which may inhibit maturation. Pre in vitro oocyte maturation, Petri-dishes were incubated in CO2 incubator (5% CO2) at 38.5°C and high humidity for 1 hour as equilibration period. About 200 µl of maturation medium was placed into sterile Petri-dish (30 x 60 mm) and covered by sterile mineral oil (Sigma Chemi. Co.), then 10-20 oocytes were allocated by pasture pipette and cultured.

After 24 h incubation of Petri-dishes in 5% CO2 incubator at 38.5°C and high humidity, only compact cumulus oocytes (COCs) were taken from the maturation medium and the cumulus cells on the oocyte surface were isolated by repeated pipetting. Thereafter, 10-20 oocytes in medium were pippeted and mounted into glass tube 5 ml well-closed and containing fixative solution (3 ml) for 24-48 h. After that oocytes mounted into glass slide, A cover slip with inert paraffin wax spots at each of its four corners was placed directly over the center of drop of the fixative containing oocyte.

Thereafter, oocytes were examined under microscope by high magnification; the cover slip was pressed down on the oocyte until it was held firmly in place. Both low power and oil immersion were used for detailed examination.

After in vitro maturation, oocytes were characterized according to Sirard *et al*. (1989) at different maturation stages, including germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (M I), metaphase Π (M Π) and degenerated oocytes.

Statistical Analysis:

General linear model of SAS (2001) was used for statistical analysis of the obtained data (ANOVA) as two ways design (2 season x 2 reproductive statuses)

for study the effect of season, bearing CLs and their interaction.

The percentages values were adjust to arcsine transformed before performing the ANOVA. Means were presented after being recalculated from transformed values to percentages.

RESULTS AND DISCUSSION

Ovarian characteristics:

Among ovarian characteristics, only ovarian weight higher (P<0.05) in breeding than in non-breeding season and with ovaries with than without CLs. However, ovarian length and thickness was affected (P<0.05) affected only by season, being longer and thicker in breed in than in non-breeding season. Generally, ovaries with CLs during the breeding season showed the highest ovarian characteristics. In addition, number of all visible follicles/ovary tended to be higher in breeding than in non-breeding season and of ovaries with than without CLs, but the differences were not significant (Table 1).

It is of interest to note that increasing the ovarian weight in breeding season is associated with longer and thicker ovaries in breeding season, but this increase not related to number of ovarian follicles.

In accordance with the effect of bearing CL on the ovaries of sheep, Khandoker *et al.* (2001) observed the same trend in buffaloes. The ovarian weight was higher (P<0.05) on ovaries bearing CL than in non-bearing ovaries. Also, Gupta *et al.* (2003) found marked differences (P<0.05) in the ovarian weight of bearing or non-bearing CL ovaries.

Concerning the ovarian measurements, Ramsingh *et al. (*2013) reported that ovarian diameters play an important role on oocyte grading and recovery rate in goat. The ovarian size variation is associated with breeding season (Davachi *et al.* 2014). There were remarkable differences in timing of the breeding season between breeds, and even between individual animals for the same breed, zone location (latitude) (Zarazaga *et al.,* 2004).

Table 1. Ovarian characteristics of ewes as affected by breeding season, CLs bearing and their interaction.

Item	CL bearing	Breeding season	Non-breeding season	Overall mean
Ovarian weight (g)	With CL	1.13±0.08	0.79±0.06	0.96±0.05[a]
	Without CL	0.41±0.03	0.44±0.03	0.42±0.02[b]
	Overall mean	0.78±0.41[a]	0.62±0.04[b]	-
Ovarian length (cm)	With CL	1.41±0.07	1.21±0.06	1.310±0.05
	Without CL	1.10±0.03	0.95±0.03	1.023±0.02
	Overall mean	1.25±0.04[a]	1.08±0.04[b]	-
Ovarian thickness (cm)	With CL	1.13±0.06	0.9±0.05	1.01±0.04
	Without CL	0.8±0.03	0.68±0.03	0.74±0.02
	Overall mean	0.96±0.32[a]	0.79±0.03[b]	-
Ovarian width (cm)	With CL	0.76±0.07	0.63±0.07	0.70±0.08
	Without CL	0.63±.030	0.59±0.02	0.61±0.03
	Overall mean	0.69±0.03	0.61±0.03	-
Number of follicles/ovary	With CL	7.04±0.74	6.89±0.86	6.97±0.57
	Without CL	5.48±0.74	5.53±0.74	5.51±0.53
	Overall mean	6.26±0.53	6.21±0.57	-

Means denoted with different superscripts in the same row or column for each factor are significantly different at P<0.05.

The previous findings and those obtained in our study indicated cyclicity of ewes in breeding season. In this respect, Kachiwal *et al*. (2012) average weight of ovaries with CL increased by advancing pregnancy period in pregnant Kundhi buffaloes. Also, Neelam and Saigal, 2005) reported that buffalo ovarian weight, volume, length and breadth, but not for the thickness, were higher during luteal phase as compared to other reproductive cycle.

Yield and recovery rate of oocytes:

Results in Table (2) revealed significant (P<0.05) effect of breeding season only on oocyte yield per ovary, being greater by 41.7% in breeding than that in non-breeding season. Also, oocyte yield was greater on ovaries with CLs than those on ovaries without CLs, but the differences were not significant. Oocyte recovery rate was insignificantly higher in breeding and on ovaries with CLs than in non-breeding season and on those without CLs.

As proved in our study, several investigators indicated higher oocyte yield in breeding than in non-breeding season. In this respect, Dadashpour *et al*. (2014) found that the number of goat oocytes recovered by different collection methods was higher in breeding than in non-breeding season. Also, Majeed *et al*. (2015) reported that recovery rate was higher (87.3%) in breeding season than in non-breeding one (72.5%). Similar observations had been reported by several investigators *Davachi et al*. (2014). In accordance with the tendency of increasing oocyte recovery from ovaries with CLs, Dode *et al*. (2001) found higher oocyte yield in non-pregnant cows in dry season. Improving oocyte yield in breeding season may attributed to the presence of more follicles during the breeding season. Generally, the highest number of recovered oocytes might reflect the optimum level of gonadotropins and steroids in breeding season (Dadashpour *et al*., 2014). In sheep, some authors have suggested that seasonal anoestrous in non-breeding season can adversely affect the number of recovered oocytes per female (Stenbak *et al*., 2001; Vázquez *et al*., 2009).

Contrary, Farag *et al*. (2010) revealed that the total numbers of recovered oocytes per ovary in Egyptian sheep were aspirated during spring than those collected during winter, summer and autumn. Cognie *et al*. (1999) revealed insignificant differences between numbers of recovered goat oocytes in breeding and non-breeding seasons. Roa *et al*. (2002) reported that the number and quality of oocytes obtained not support differential folliculogenic activity of the ovaries during the breeding and non-breeding season.

In accordance with the effect of bearing CLs on increasing oocyte yield, Mara *et al*. (2013) showed that the ovaries collected during the breeding season generally had

well developed CL and most of the follicles were larger than 2–3 mm. Contrarily Huma *et al.* (2008) showed significantly greater population of buffalo oocytes per ovary from ovaries without CL than those with CL. However, Freistedt *et al.* (2001) found that the ovaries collected during the anoestrus season were smaller, had smaller follicles, and no CLs were recorded. These data are similar to those reported for seasonal breeder animals.

According to Nandi *et al.* (2000), the oocytes recovery rate decreased when ovaries bear CL because lutein cells occupy most of the ovary, so follicular development is restricted. On the other hand, Torner *et al.* (2003) reported that no significant differences among the distribution of recovery COCs types (compact, depressed, corona radiate and denuded oocytes) from non-pregnant and pregnant camel donor.

Table 2. Yield and recovery rate of sheep oocytes as affected by breeding season, CLs bearing and their interaction.

Item	CL bearing	Breeding season	Non-breeding season	Overall mean
Oocytes/ovary	With CL	5.17±0.79	3.61±0.97	4.50±0.64
	Without CL	4.04±.066	3.00±0.37	3.54±0.40
	Overall mean	4.62±0.52[a]	3.26±0.43[b]	-
Recovery rate (%)	With CL	72.98±2.08	58.24±20.6	66.66±8.41
	Without CL	73.05±9.22	56.54±8.05	64.80±6.47
	Overall mean	73.01±4.37	57.27±8.91	-

Means denoted with different superscripts in the same row or column for each factor are significantly different at P<0.05.

Oocyte category:

Results in Table (3) showed that number/ovary and frequency distribution of compact cumulus complex (COCs) and degenerated oocytes were higher, while number/ovary and frequency distribution of denuded and partial denuded oocytes were lower in breeding than in non-breeding season. As affected by presence of CLs, number and frequency distribution of all categories were higher for oocytes recovered from ovaries with than without CLs. It is of interest to note that the significant (P<0.05) differences were observed only for number of degenerated oocytes/ovary and for frequency distribution of denuded oocytes as affected by season.

Table 3. Number/ovary and frequency distribution of sheep oocyte categories as affected by breeding season, CLs bearing and their interaction.

Item	CL bearing	Breeding season	Non-breeding season	Overall mean
Number of oocyte category/ovary:				
Compact cumulus complex	With CL	2.24±0.21	1.78±0.89	2.14±0.38
	Without CL	2.27±0.55	1.39±0.19	1.83±0.32
	Overall mean	2.34±0.27	1.56±0.36	-
Partial denuded	With CL	0.29±0.24	0.44±0.44	0.36±0.21
	Without CL	0.20±0.14	0.42±0.14	0.31±0.10
	Overall mean	0.25±0.13	0.43±0.18	-
Denuded	With CL	0.29±0.17	0.83±0.33	0.52±0.19
	Without CL	0.30±0.15	0.55±0.27	0.42±0.15
	Overall mean	0.30±0.11	0.67±0.20	-
Degenerated	With CL	1.67±0.53	0.56±0.06	1.19±0.36
	Without CL	1.29±0.26	0.65±0.30	0.97±0.22
	Overall mean	1.48±0.28[a]	0.61±0.16[b]	-
Frequency distribution of oocyte category (%):				
Compact cumulus complex	With CL	49.89±8.21	45.45±16.17	47.99±7.58
	Without CL	53.51±5.61	49.81±10.77	51.66±6.42
	Overall mean	51.7±5.55	47.94±8.44	-
Partial denuded	With CL	4.89±3.37	9.52±9.52	6.87±4.13
	Without CL	4.30±2.3	13.66±3.71	8.98±2.68
	Overall mean	4.6±1.89	11.88±4.19	-
Denuded	With CL	5.61±3.35	24.42±6.68	13.67±4.90
	Without CL	6.13±2.91	15.69±7.17	10.91±4.01
	Overall mean	5.87±2.06[b]	19.43±4.92[a]	-
Degenerated	With CL	32.47±9.15	20.61±9.70	27.39±6.57
	Without CL	35.66±10.89	20.84±7.87	28.5±6.82
	Overall mean	34.07±6.61	20.74±5.58	-

Means denoted with different superscripts in the same row or column for each factor are significantly different at P<0.05.

One One of the most important factors that can influence the success of the IVEP is the efficient recovery of the cumulus-oocyte complexes (COC's) (Keskintepe *et al.* 1998). It is worthy noting that yield and frequency distribution of oocytes arrested at compact stage was the highest as compared to other stages, regardless season or CL bearing. Rezk *et al.* (2005) demonstrated that oocyte frequency distribution with >4 cumulus cell layers was the highest and of fragmented oocytes was the lowest as compared to the other oocyte categories. Shamiah (2004) found that number and distribution of buffalo oocytes with >3 layers were the highest, regardless CL presence.

The observed greater yield and proportion of compact oocytes in breeding than in non-breeding was reported in camels by several authors. In this way, Farag *et al.* (2010) recorded higher proportion of COCs and improved oocyte yield/ovary in sheep during spring than in winter and summer. In goats, the highest recovered oocyte yield was found in the breeding season, irrespective of recovery technique (Dadashpour *et al.*, 2014). In sheep, COCs percentage was lower (P<0.05) in winter than in summer (Seydou *et al.*, 1999; Gou *et al.*, 2009). In camels, Abdoon (2001) showed that yield of compact cumulus cells was lower (P<0.01) in non-breeding than in breeding season.

Amer (2004) found more excellent and good oocytes (P<0.01) during the breeding season than in non-breeding one. However, the incidence of degenerated or very bad oocytes was not influenced by season. Improving recovery of goat oocytes in the breeding season may reflect release of sufficient concentration of gonadotrophins and steroid hormones during the breeding season (Dadashpour *et al*., 2014). On the other hand, Cognie *et al*. (1999) reported insignificant effect of breeding season on yield of recovered oocytes in sheep and goats.

In agreement with the present insignificant effect on yield and distribution of oocytes recovered from ovaries with or without CLs, Hazeleger *et al*. (1995) reported that presence of CL on large follicles had no effect on the number of cumulus oocytes complex in bovine. In accordance with the tendency of higher oocyte production from ovaries with Cls, Abdoon and Kandil (2001) showed that buffalo ovaries bearing CL had a significantly (P<0.05) higher number of good quality oocyte than ovaries non-bearing CL. Contrary, buffalo oocytes yield from CLs bearing ovaries significantly (P<0.01) decreased as compared to non-CL bearing ovaries (Das *et al*., 1996). Also, ovaries bearing CL had significantly (P<0.05) lower mean number of oocytes as compared to those without CL (Amer *et al*., 2008). Dode *et al*. (2001) found that the percentage of usable oocytes was higher in non-pregnant cows in dry season. Also, Abdoon (2001) showed that the number of camel oocytes (≥5 layers of compact cumulus cells) was significantly (P<0.01) greater in ovaries without CL 9.1 oocytes/ovary than with CL 5.7 oocytes/ovary, respectively.

Generally, marked interaction between ovaries with CL and breeding season, physiological status and feeding system was obtained by Shamiah (2004) in buffaloes. During the breeding season, ovaries generally had well developed and functional CLs and follicular size more than 2–3 mm (Mara *et al*., 2013). Meanwhile during non-breeding season, ovaries had smaller follicles without CLs (Freistedt *et al*., 2001).

Maturation rate:

Effect of season:

Results in Table (4) showed that percentage of oocytes at M-I stage was slightly higher in non-breeding than in breeding season. However, percentage of oocytes at M-II showed an opposite trend, reflecting higher maturation rate (M-I and M-II) in breeding than in non-breeding season, but the difference was not significant.

In accordance with the present results, Davachi *et al*. (2014) reported insignificant differences in the percentage of sheep oocytes at M-II stage that were recovered in breeding (79.3%) and non-breeding (76.7%) seasons, being higher in comparable with our results in the present study (52.16 vs. 48.42%). In goats, Majeed *et al*. (2015) showed maturation rate of 45.6% in breeding season as compared to 35.2% in non-breeding season, being lower than those reported on sheep in our study (67.02 vs. 65.13%). Moreover, Rao *et al*. (2002) have been found that there was no difference in efficiency of IVM of oocytes collected during the breeding and non-breeding season in sheep.

In camels, Zeidan *et al*. (2011) found that full expanded cumulus complexes oocytes (after 24 h of maturation) was higher (P<0.05) in breeding season than in the non-breeding season (80.7 vs. 66.5%). Amount of follicular estrogens produced by the ovaries may differ in breeding season from that in non-breeding one (Kaushish, 1994). Alternatively, antral follicles on the non-breeding season ovaries did not reach to the ovulatory volume to produce sufficient estrogen. So, the oocyte ability to undergo IVM was not affected by the season, but was dependent on the maturation medium (Rao *et al.,* 2002, Kaushish *et al.,* 2011).

Table 4. Frequency distribution of sheep oocytes at different *in vitro* maturation stages as affected by breeding season.

Stage of mature oocyte		Breeding season (N=384)	Non-breeding season (N=370)
Germinal vesicles	N	26	18
	%	6.83±0.76	5.04±0.83
Germinal vesicles breakdown	N	28	28
	%	6.26±1.003	7.58±1.10
Metaphase-I	N	61	62
	%	15.52±1.57	16.68±1.73
Metaphase-II	N	196	157
	%	52.16±2.47	48.42±2.7
Maturation rate	N	257	237
	%	67.02±1.95	65.13±2.14
Degenerated	N	73	87
	%	18.37±1.42^{b}	22.5±1.48^{a}

Means denoted with different superscripts in the same row or column for each factor are significantly different at P<0.05.

Effect of type of serum in maturation medium:

Results in Table (5) showed that percentage of oocytes at M-I and M-II stages was higher in BSA medium than in sheep serum medium, but the differences were not significant.

Table 5. Frequency distribution of sheep oocytes at different *in vitro* maturation stages as affected by type of serum in maturation medium.

Stage of mature oocyte		Bovine serum albumin (N=348)	Sheep serum albumin (N=406)
Germinal vesicles	N	19	25
	%	5.78±0.79	6.25±0.79
Germinal vesicles breakdown	N	25	31
	%	6.79±1.05	7.15±1.05
Metaphase-I	N	50	73
	%	14.55±1.65	17.55±1.65
Metaphase-II	N	172	199
	%	49.68±2.85	50.64±2.85
Maturation rate	N	222	272
	%	64.13±2.49	68.19±2.49
Degenerated	N	82	78
	%	22.45±1.47	18.04±1.47

These results may reflect slight improvement on maturation rate of sheep oocytes as affected by sheep serum addition. Adding serum to the culture medium of oocytes provides albumin source that balances the osmolarity and acts as a free radical scavenger (Thompson, 2000). Bovine serum albumin (BSA) was used as the main protein source for IVM of oocyte in different animal species (Gordon, 1994). Sheep oocytes have also been studied for different types of maturation media (Wani, 2002; Roa *et al*., 2002), supplemented with FBS (fetal bovine serum) as reported by Wani *et al*. (2000), ESS (estrous sheep serum) as reported by Ghasemzade-Nava and Tajik (2000) and human serum as reported by Thomson *et al*. (1992) as protein

supplementation. Different results were obtained with serum supplementation.

In sheep, Braun (1988) found that FCS had ability to support oocyte cumulus expansion as compared to BSA. However, El-Maghraby (2004) found no effect of BSA and estrus ewe serum addition to TCM-199 on oocyte maturation rate as compared to the control medium. Also, Farag *et al*. (2010) revealed that SS addition to TCM-199 had no impact on IVM of denuded oocytes.

In most experiments, addition of sera of the same animal species was more beneficial than those of other species on IVM of oocytes. This was proved by rabbit serum addition to TCM-199 than BSA on IVM of rabbit oocytes, in cattle, Leibfried-Rutledge *et al*. (1987) found that fetal calf serum (FCS) is necessary for FSH-induced cumclus-cell expansion and also improved cumulus-cell viability and completion of the first meiotic division in compact oocytes when compared to BSA. Also, Chen *et al*. (1994) suggested that addition of FCS or FBS to culture media led to enhance cumulus expansion of bovine oocytes. Yet, Zheng and Sirard (1992) reported inhibition of the maturation of porcine oocyte by BSA addition to the maturation media. Similarly in goats, Pawshe *et al*. (1996) found that TCM-199 supplemented with estrous goat serum (EGS) or fetal calf serum (FCS) had ineffective improving the in vitro maturation.

Effect of interaction:

Effect of interaction between season and type of serum on maturation rate was insignificant reflecting higher maturation rate of oocytes with sheep serum than with bovine serum in breeding and non-breeding season (Fig. 1).

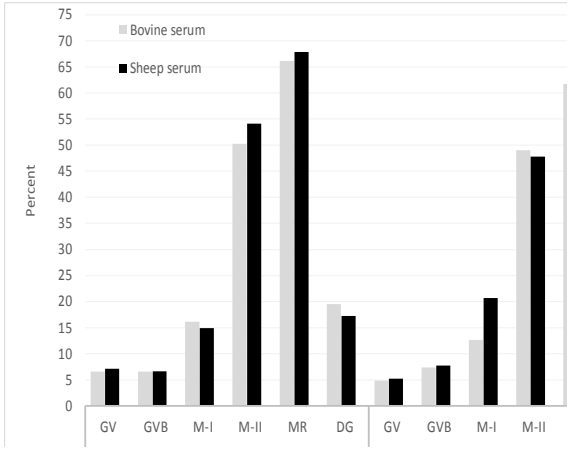

Fig. 1. Frequency distribution of sheep oocytes at different *in vitro* maturation stages the interaction between breeding season and type of serum in maturation medium.

Oocyte maturation is a complex process during which the oocyte progresses from the diplotene to M-II stage. The transition from the diplotene stage to metaphase is called diakinesis. The oocyte resumes meiosis in response to the ovulatory LH surge (Peng *et al*., 1991) or removal from the follicle (Pincus and Enzmann, 1935). The IVM rates of 69-72% in different level of serum were lower compared to published reports (Roa *et al*., 2002; Ghasemzadeh-Nava and Tajik, 2000; Wani, 2000, 2002). As affected by season, Majeed *et al*. (2015) maturation rate of goat oocytes was 45.6% in breeding season compared with 35.2% in non-breeding season. Similar trends had been reported by several investigators (Davachi *et al*., 2014).

The role of type of sera was found to relate to its contents of proteins (globulin and futuin), type of hormones, some trace nutrients (Hsu *et al*., 1987). In this line, Thompson (2000) observed that serum addition to the culture medium acts as albumin source to balances the osmolarity level and scavenging the free radicals.

As affected by CL-bearing (breeding season) and supplementation of TCM-199, El-Harairy *et al*. (2006) reported the highest maturation rate (MR) of camel oocytes in TCM-199 supplemented with fetal calf serum vs. BSA

Based on the foregoing results, sheep oocytes were available to be harvested during non-breeding season from slaughtered ewes with acceptable yield, quality and in vitro maturation rate. Adding sheep serum (10%) as alternative of bovine serum in TCM-199 medium may be have beneficial effect on maturation rate of sheep oocyte during breeding and non-breeding seasons. Further studies are required to study maturation rate of sheep oocytes as affected by addition of different levels and types of sera to different types of maturation media during non-breeding season.

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تأثير الحالة التناسلية على إنتاج وانضاج بويضات الأغنام المصرية

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تهدف هذه الدراسه لمعرفة تأثير موسم التناسل و وجود الجسم الاصفر على نشاط المبيض بالإضافة إلى تأثير موسم التناسل وإضافة سيرم الأغنام وسيرم البيومين مصل الأبقار على معدل الإنضاج المعملي لبويضات الاغنام المصريه. بعد جمع المبايض من المجازر الاهلية من برج العرب- الأسكندرية تم اخذ قياسات المبيض (وزن , طول, عرض وسمك) ثم تم جمع البويضات بإستخدام طريقة التشريح و حساب معدل الاسترداد.بعد ذلك تم زراعة البويصات الجيده في بيئة زراعة الأنسجه (TCM199) المضاف اليها سيرم الأغنام او البيومين مصل الأبقار خلال موسمي التناسل.لوحظ زيادة وزن المبيض (جم) بدرجة معنوية , طول وسمك المبيض بدرجة غير معنوية أثناء موسم النشاط الجنسي وفي وجود الجسم الاصفر وذلك بالمقارنة بموسم الخمول الجنسي وفي غياب الجسم الاصفر. لوحظ ايضا زياده في عدد الحويصلات المبيضية بدرجة معنوية أثناء موسم النشاط الجنسي وفي وجود الجسم الاصفر. كان معدل الاسترداد وانتاج البويضات أثناء موسم النشاط الجنسي عالي بدرجة غير معنوية وفي وجود الجسم الاصفر وذلك بالمقارنة بموسم الخمول الجنسي وفي غياب الجسم الاصفر.لوحظ ايضا متوسط عدد البويضات عالية الجودة كانت أعلى بدرجة غير معنوية أثناء موسم النشاط الجنسي وذلك بالمقارنة بموسم الخمول الجنسي وفي غياب الجسم الاصفر. زاد معدل الإنضاج المعملي أثناء موسم النشاط الجنسي للبويضات التي وصلت لمرحلة metaphase II بدرجة غير معنوية وذلك بالمقارنه بموسم الخمول الجنسي بغض النظر عن كل من نوع البيئه و السيرم المستخدم, كذلك زاد معدل الإنضاج المعملي للبويضات التي وصلت لمرحلة metaphase II غير معنوية مع سيرم الاغنام (SS) وذلك بالمقارنه مصل سيرم الأغنام. نستخلص من هذه الدراسه امكانية اجراءالانضاج المعملي للبويضات المسترده اثناء موسم النشاط الجنسي. كذلك يمكن تحسين معدل الإنضاج المعملي للبويضات التي يتم جمعها في غير موسم التزاوج بإضافة سيرم الاغنام إلى بيئة الإنضاج.