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Ovarian Biometry, Oocyte Yield and Oocyte Quality of Baladi Goats as Affected by Ovarian Status in Breeding and Non-Breeding Season

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ABSTRACT

The current study was undertaken to elucidate the effect of ovarian status in breeding and non-breeding season on the ovarian biometry, oocyte yield and oocyte quality of Baladi goats. Ovaries were collected by slicing from slaughter houses and classified with or without CLs during breeding (September-December) and non-breeding (March-July) seasons. Ovaries were weighed and measured, while oocytes were recoverded, yielded and categorized with or without CLs in breeding or non-breeding seasons. Results showed that ovarian weight and biometry (length, width and thickness) were higher in breeding than in non-breeding season, but the differences were significantly only for width. Number of follicles and oocytes/ovary ($P<0.001$) as well as number/ovary and proportion of oocytes at compact ($P<0.0001$) and denuded ($P<0.05$) stage were higher in breeding season than in non-breeding one. Number of degenerated oocytes/ovary was not affected significantly by season, but its proportion was lower ($P<0.001$) in breeding than in non-breading season. Number/ovary and proportion of partial denuded oocytes and proportion of denuded oocyte were not affected significantly by breeding season. Weight and biometry of ovaries was higher on ovaries bearing CL (CL+) than in non-bearing ones (CL-). Only ovarian width was higher ($P<0.001$) by 38% in CL+ than in CL- group. Ovaries bearing CL had higher ($P<0.05$) total follicles and oocyte yield/ovary ($P<0.01$) as well as oocyte recovery rate ($P<0.05$) than CL- ovaries. Number of compact, denuded and partial denuded oocytes/ovary was not affected by CL bearing. Number of compact oocytes tended to be greater on ovaries without than with CL. Number of degenerated oocytes/ovary was higher ($P<0.05$) on CL+ ovaries. Proportion of all oocyte categories was not affected by bearing CL. Finally, the effect of interaction between breeding season and bearing CL on all parameters studied was not significant. In conclusion, the goat ovaries without CL in breeding season yielded better oocyte quality than in non-breeding season, in term of COCs proportion. During non-breeding season, goats oocytes were available to be harvested from slaughtered goat does with acceptable yield and quality.

Keywords: *Goats, seasonality, oocytes, progesterone.*

INTRODUCTION

For genetic improvement, it is important to develop *in vitro* embryo production, *IVEP* (Brackett *et al*., 1982). It is well known that oocyte *in vitro* maturation *(IVM)* and fertilization *(IVF)* as well as embryo culture (EC) are vital steps for *IVEP* (Sirard *et al.,* 1989). The preliminary goal of assisted reproductive technologies (ART) is to rapidly gain genetic improvement of livestock (Baldassarre *et al*., 2003) as well as transgenic and cloning (Li *et al*., 2006).

The seasonal effect on *IVEP* in various animal species was studied (Colleoni *et al*., 2004; Silva *et al.,* 2006). In cattle of subtropical area, the season effect on *IVEP* was not demonstrated (Rivera *et al.*, 2000), while blastocyst yield differ in the cold compared with hot season was observed in Egyptian buffalo (Khairy *et al.*, 2007) as seasonally polyestrous animals.

Goats are seasonal breeders (Hafez and Hafez, 2000) and season has important role on reproductive performance of ovine (Carolan *et al.,* 1994). It was reported that oocyte grading and recovery rate (Ramsingh *et al*., 2013) were affected by ovarian characteristics in different seasons and under various nutritional systems. There are some deficiencies in *IVM-IVF* systems in non-breeding season compared with breeding one. Therefore, improving *IVEP* during non-breeding season is required by optimizing the IVM system (Carolan *et al.,* 1994). In seasonal anestrous sheep, oocytes yield, developmental competence, and the IVF differed from that in breeding season (Stenbak *et al.,* 2001; Vázquez *et al.*, 2009). In cat during non-breeding season, oocyte IVM reached 20%, without embryo development to the blastocyst stage (Spindler and Wildt, 1999).

Corpus luteum (CL) is necessary for progesterone (P4) production, 1) throughout the luteal phase of the estrous cycle to maintain pregnancy if a conceptus is present, and 2) during pregnancy, to decrease gonadotrophin secretion and prevent behavioral estrous occurring (Mann *et al*., 1998; Powell *et al*., 2006). In addition, this small, transient endocrine gland secrets small quantities of oestradiol-17ß, prostaglandins and a number of peptide hormones such as relaxin, oxytocin, oxytocin-related neurophysin-I, vasopressin and inhibin (Field, 1991). A relationship between the development of CL and the development of follicles, offers new evidence to support the existence of factors associated with heterogeneity in the developmental competence of oocytes (Contreras-Solis *et al*., 2008). Effect of bearing CL in bovine (Sugulle *et al*., 2008) and breeding season in sheep and goats (Cognie, 1999) was studied on oocyte yield and quality.

Therefore, the present study was undertaken to elucidate the effect of ovarian status in breeding and non-breeding season on the ovarian biometry, oocyte yield and oocyte quality of Baladi goats.

MATERIALS AND METHODS

This study was conducted at the International Livestock Management Training Center (ILMC), 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 September 2015 to January 2018.

Ovaries were collected from private slaughter houses (Borg El-Arab, Alexandria Province, about 225 km from the laboratory) during breeding season interval from September to December, and during non-breeding season interval from March to July. Ovaries were collected immediately after slaughtering. Ovaries were placed in punctured plastic bag and stored into thermos containing saline solution (0.9% NaCl, 100 IU penicillin and 100 µg streptomycin/ml) at 28°C. Ovaries were transported to the laboratory within 3 hours.

In the laboratory, all excess tissues from the stalk of the collected ovaries were isolated, then ovaries were washed two times with warmed (28°C) PBS (phosphate buffer solution) supplemented with 100 IU penicillin and 100 µg streptomycin/ml to clean adhering clotted blood.

Thereafter, ovaries were quickly washed one time with 70% ethanol for removing any contamination on the ovarian surfaces.

Ovaries of breeding or non-breeding season were classified into ovaries bearing corpus luteum (CL) and ovaries without CL. Ovaries were weighed, then their length, width and thickness were determined using caliper.

Harvesting medium included one PBS sigma tablet dissolved in 200 ml sterile distilled water with penicillin (100 IU) and streptomycin (100 µg) per ml, and bovine serum albumin (2 mg/ml). Harvesting medium was adjusted to 7.2-7.4 pH (pH-meter) and 280-300 mOsmol/kg (osmometer), and filtered by 0.22 µm-millipore (Milieux GV, Millipore, Cooperation Bedford, and MOA).

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 from each ovarian type were washed 3 times in the harvesting medium, and then oocytes were searched by stereomicroscopy and classified into four categories. According to Madison *et al*. (1992), oocytes were classified into 1) oocytes with 5 or more cumulus cell layers (compact cumulus complex, COCs), 2) oocytes with incomplete cumulus cell layers (partial denuded), 3) oocytes without cumulus cell layers attaching zona pellucida (denuded), and 4) oocytes with shrunken or incomplete ooplasm away from the zona pellucida, or with empty zona pellucida (degenerated).

Data were statistically analyzed using General Linear Model procedures of SAS (2001) in factorial design to study the effect of season (breeding and non-breeding), CL (bearing and non-bearing) and their interaction on ovarian biometry, and yield and quality of oocytes.

RESULTS AND DISCUSSION

Effect of breeding season:

Ovarian weight and biometry:

Ovarian weight and all biometric characteristics were higher in breeding than in non-breeding season, but only ovarian width was significantly higher (P<0.001) in breeding than in non-breeding season. However, ovarian weight, length and thickness tended to be higher in breeding than in non-breeding season, but the differences were not significant (Table 1).

Table 1. Effect of breeding season on ovarian weight and biometry in goat does.

Ovarian biometry	Season		P-value
	Breeding	Non-breeding	
Weight, g	0.92±0.09	0.74±0.11	0.232^{NS}
Length, cm	1.32±0.061	1.16±0.07	0.114^{NS}
Thickness, cm	0.90±0.05	0.80±0.06	0.321^{NS}
Width, cm	0.85±0.05	0.60±0.06	0.000***

NS: Not significant. * Significant at P<0.001.**

In seasonal animals, timing the breeding season is different among breeds, individuals and zone location (Zarazaga *et al.,* 2004). The recorded higher ovarian biometry, especially ovarian width, P0.001) in breeding than in non-breeding season may indicate lower ovarian activity of local goats in non-breeding season from March-July in Egypt.

The remarkable higher ovarian biometric measurements in breeding than in non-breeding season may be attributed to presence of more ovarian structure in breeding than in non-breeding season. In ovine, breeding season is associated with increasing the ovarian measurements (Davachi *et al*. 2014), whereas ovaries collected during breeding season were bigger with larger follicles and CLs in comparing with tose collected in non-breeding season (Freistedt *et al*., 2001).

Oocyte recovery:

Number of follicles and oocytes per ovary was affected significantly (P<0.001) by breeding season, being greater by 54.70 and 47.55%, respectively in breeding than in non-breeding season. This was reflected in nearly similarity in oocyte recovery rate in both seasons (85 *vs*. 83%, Table 2). It is worthy noting that the observed significant increase in ovarian width was in association with increasing follicular number.

Increasing yield of oocytes in breeding than in non-breeding season was in association of increasing ovarian biometry, and was mainly related to numerous follicles containing oocytes during breeding season as compared to non-breeding season. Generally, ovarian size had marked effect on recovery rate of oocytes in goats (Ramsingh *et al*., 2013).

Table 2. Effect of breeding season on recovery of goat doe oocytes.

Biometry	Season		P-value
	Breeding	Non-breeding	
Number of total follicles/ovary	6.61±0.19	4.37±0.19	0.0001***
Number of oocytes/ovary	5.71±0.26	3.87±0.25	0.0001***
Recovery rate	84.0±3.12	83.0±2.54	0.855^{NS}

NS: Not significant. * Significant at P<0.001.**

Similar to the obtained results, yield of oocyte (Davachi *et al.,* 2014) and oocyte recovery rate (Majeed *et al.,* 2015) was higher in breeding season than in non-breeding one of goats. Also, Stenbak *et al*. (2001) and Vázquez *et al*. (2009) in sheep showed adverse effects of non-breeding season on yield of recovered oocytes. As affected by month of the year, yield of recovered oocytes were aspirated from ewes in spring than in winter, summer and autumn (Farag *et al*., 2010). In general, enhancing yield of recovered oocytes in our study in the breeding season may be attributed to releasing sufficient gonadotrophins and steroid hormones levels in the breeding season as compared to non-breeding one (Davachi *et al.,* 2014).

On the other hand, some investigators found insignificant effect of season on yield of recovered oocytes in goats (Cognie, 1999).

Oocyte category:

Data in Table (3) showed that doe ovaries significantly yielded greater number of cumulus-oocyte complexes (COCs, P<0.0001) and denuded oocytes (P<0.05) as well as higher proportion of COCs (P<0.001) per ovary in breeding season than in non-breeding one.

Although number of degenerated oocytes per ovary was not affected significantly by season, its proportion was significantly (P<0.001) lower in breeding than in non-breading season. However, number and proportion of partial denuded oocytes and proportion of denuded oocyte were not affected significantly by breeding season.

Table 3. Effect of breeding season on oocyte categories per ovary of goats.

Item	Season		P-value
	Breeding	Non-breeding	
Oocyte number per ovary:			
Cumulus-oocyte complexes	2.81±0.28	1.27±0.06	0.0001***
Partial denuded/ovary	0.41±0.10	0.45±0.09	0.3415^{NS}
Denuded/ovary	0.86±0.11	0.51±0.04	0.0288*
Degenerated/ovary	1.60±0.12	1.71±0.21	0.4479^{NS}
Frequency distribution (%):			
Cumulus-oocyte complexes	49.32±3.35	33.39±1.81	0.0025**
Partial denuded	7.5±2.26	12.9±3.92	0.3200^{NS}
Denuded	15.13±1.84	13.39±1.15	0.4919^{NS}
Degenerated	28.20±1.98	42.82±3.65	0.0023**

NS: Not significant. * Significant at P<0.05. ** Significant at P<0.01. * Significant at P<0.001**

Results of oocyte quality are in matching with ovarian biometry and oocyte recovery in both seasons. In this respect, Ramsingh *et al*. (2013) observed the Importance of diameter of the ovary in grading of recovered oocytes in goat. One of the most important factors that can influence the success of the IVEP is the efficient recovery of COCs (Keskintepe *et al.*, 1998). It is worthy noting that COCs were the highest as number and proportion as compared to other stages in breeding season.

However, degenerated oocytes represented the same situation in non-breeding season. In buffalo, Shamiah (2004) found the greatest number and the highest frequency distribution of oocytes arrested with more than three layers, bearing CLs.

Increasing distribution of COCs in breeding season was observed in goats, which showed the highest yield and frequency distribution in the breeding season (Davachi *et al.,* 2014). In sheep, Farag *et al.* (2010) observed that COCs showed lower yield per ovary in winter and summer than in spring, and in winter than in summer (Seydou *et al.,* 1999; Gou *et al*., 2009). In camels, COCs yield (Abdoon, 2001), and excellent and good oocytes were higher in breeding season than in non-breeding one.

Effect of CL(s) bearing:

Ovarian weight and biometry:

Although weight and biometry of doe ovaries was higher on ovaries bearing CL (CL+) than in non-bearing ones (CL-), only ovarian width was significantly (P<0.001) was higher by 38% in CL+ than in CL- group (Table 4). The tendency in ovarian weight was mainly due to increasing ovarian width on ovaries bearing CL.

Table 4. Effect of bearing ovaries CL on ovarian weight and biometery of goat does.

CL bearing	CL+	CL-	P-value
Weight, g	0.95±0.05	0.71±0.02	0.116^{NS}
Length, cm	1.30±1.08	1.18±0.05	0.229^{NS}
Thickness, cm	0.89±0.04	0.82±0.02	0.507^{NS}
Width, cm	0.84±0.07	0.61±0.04	0.004**

NS: Not significant. ** Significant at P<0.01.

In goats, the differences in the ovarian weight between of bearing and non-bearing CL ovaries were reported by Gupta *et al.* (2003), being significantly heavier when ovaries bear CL than in non-bearing ovaries (0.71 vs. 0.64 g), while the mean length was higher in the ovaries without CL. In buffaloes, Neelam and Saigal (2005) reported that weight, volume, length and breadth, but not for the thickness, were the highest on the ovaries during luteal phase. In this respect, there was a marked increase in ovarian weight by advancing pregnancy period in pregnant Kundhi buffaloes (Kachiwal *et al.,* 2012). Moreover, Asad *et al.* (2016) reported increasing ovarian width and weight in ovaries with CL than those of ovaries without CL.

These results was attributed to that the CL is an extra cellular mass within the ovary which made the differences of its width and weight. While, the ovarian length was found reverse in the ovaries without CL (Jablonka-Shariff *et al.,* 1993).

Oocyte recovery:

Results in Table (5) revealed significant (P<0.05) effect of bearing CL on total follicles and oocyte yield per ovary (P<0.01) as well as oocyte recovery rate (P<0.05), being higher in on ovaries without than with CL. These results contrary the ovarian biometry and this may be attributed to those ovaries with CL were occupied by large area with CL. Nandi *et al*. (2000) observed that oocyte recovery rate decreased in ovaries bearing CL because lutein cells occupy most of the ovary, leading to restriction of follicular development.

Table 5. Effect of bearing ovaries CL on recovery of goat doe oocytes.

CL bearing	CL+	CL-	P-value
Total follicles/ovary	4.99±0.21	5.997±0.81	0.0028**
Oocyte/ovary	$4.06±0.37^{b}$	$5.40±0.33^{a}$	0.0020**
Recovery rate	$0.79±0.01^{b}$	$0.88±0.03^{a}$	0.0379*

*** Significant at P<0.05. ** Significant at P<0.01.**

In accordance with the tendency of increasing oocyte recovery from ovaries without CL, Islam *et al*. (2007) reported the total and normal number of goat COCs was higher in CL- ovaries as compared to CL+ ovaries (1.87 and 1.12 vs. 1.76 and 0.76/ovary, respectively). This finding also have been supported by previous studies in sheep (Widyastuti *et al.,* 2017), whereas presence of CL on the ovaries adversely affected total number of follicle and consequently oocyte number. Similar trends were obtained in different species, including goats (Kumar *et al.,* 2004), sheep (Contreras-Solis *et al.*, 2008), bovine (Shabankareh *et al.*, 2015), buffalo (Huma *et al*., 2008; Makwana *et al.*, 2015) and camel (Amer *et al*., 2008). On the other hand, Torner *et al.* (2003) found insignificant effect of reproductive status (pregnant and non-pregnant) on proportion of COCs types in camels.

The trend of decreasing follicles/oocytes yield of CL+ ovaries was explained by the mechanism by which progesterone inhibits follicular growth. In this mechanism, progesterone suppressed LH pulse frequency, which is critical for growing the large follicles. In this respect, Bartlewski *et al.* (2001) suggested local effects of progesterone on development of the antral follicles during luteal and follicular phases. This may0 independent of changes in secretion of gonadotrophin.

Oocyte category:

The differences in number of COCs, denuded, partial denuded oocytes per ovary were not significant although OCOCs tended to be greater on ovaries without than with CL. Only number of degenerated oocytes per ovary was significantly (P<0.05) higher for ovaries without than with CL. On the other hand, proportion of all oocyte categories was not affecting by bear CL. The proportion of COCs was higher on CL- than CL+ ovaries, but the difference was not significant (Table 6).

Table 6. Effect of bearing ovaries CL on oocyte categories per ovary of goats.

Item	CL+	CL-	P-value
Number of oocyte category/ovary:			
Cumulus-oocyte complexes	1.67±0.23	2.41±0.37	0.1118^{NS}
Partial denuded	0.51±0.14	0.37±0.05	0.3415^{NS}
Denuded	0.58±0.08	0.77±0.11	0.3375^{NS}
Degenerated	$1.37±0.20^{b}$	$1.85±0.10^{a}$	0.0248*
Frequency distribution of oocyte category (%):			
Cumulus-oocyte complexes	40.47±2.56	42.77±4.43	0.9135^{NS}
Partial denuded	14.35±4.95	14.46±1.55	0.1654^{NS}
Denuded	14.46±4.90	14.21±1.06	0.8214^{NS}
Degenerated	33.58±4.18	35.99±3.64	0.14190^{NS}

NS: Not significant. * Significant at P<0.05.

In agreement with the obtained results, de Souza-Fabjan *et al.* (2014) found greater number of good quality oocytes recovered from ovaries without CL than with CL in sheep and goats. Also, Islam *et al*. (2007) obtained significantly higher number of normal COCs from goat ovaries without than with CL. In buffalo, Jamil *et al.* (2008) revealed that average number of good quality oocytes recovered from ovaries without CL was comparably higher to those with CL. Also, Das *et al.* (1996) found a reduction in oocytes yield from CL bearing ovaries as compared to non-CL bearing ovaries. In camels, Abdoon (2001) showed that COCs number (≥5 layers) was significantly (P<0.01) greater in ovaries without CL than with CL (9.1 vs. 5.7 oocytes/ovary). In addition, the proportion of usable oocytes was higher in non-pregnant cows than in pregnant cows (Dode *et al.* (2001). Contrary, ovaries with CL had higher yield and quality oocytes than those without CL in buffalo.

Effect of interaction:

The effect of interaction between breeding season and bear CL on all parameters studied was not significant, reflecting higher all biometric characteristics (Fig. 1) in breeding than non-breeding season, and on ovaries with than without CL, being the highest on ovaries with CL in breeding season.

The effect of interaction between breeding season and bear CL on number of follicles and oocytes per ovary and recovery rate was not significant, reflecting greater number of follicles (Fig. 2) in breeding than non-breeding season, and on ovaries without than with CL as well as higher recovery rate in breeding than in non-breeding season only for ovaries with CL (Fig. 3). The greatest follicles and oocytes were obtained from ovaries without CL in breeding season (Fig. 2), while the highest recovery rate was obtained from ovaries without CL, regardless breeding season (Fig. 3).

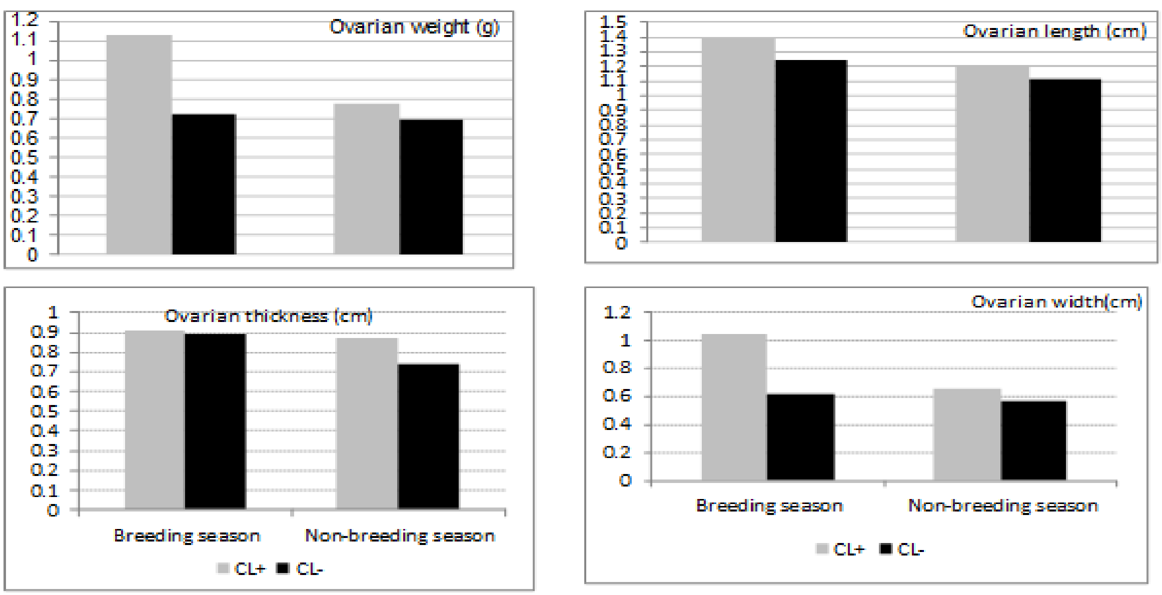

Fig. 1. Ovarian biometery on goat ovaries with or without CL during breeding and non-breeding season.

The detected insignificant interaction on frequency distribution of oocytes at different categories revealed the highest distribution of COCs in breeding season, regardless bear CL. However, during non-breeding season, distribution of degenerated oocytes was the highest, regardless bear CL (Fig. 4). This means that ovaries without CL in breeding season yielded the highest oocyte quality, in term of COCs proportion.

Similar interaction was obtained by Shamiah (2004) in buffaloes. In the breeding season, with cyclicity ovaries bear complete functional CLs and large follicles with > 2–3 mm in diameter (Mara *et al.,* 2013) versus ovaries with small follicles and without CLs (Freistedt *et al*., 2001).

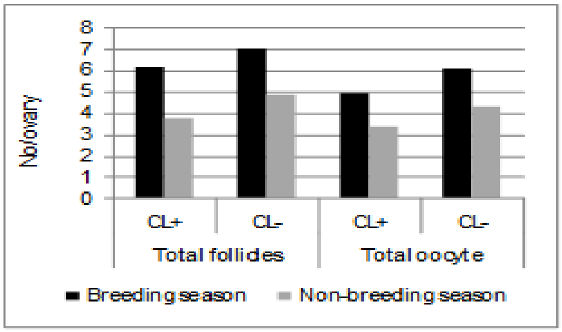

Fig. 2. Number of follicles and oocytes/ovary on goat ovaries with or without CL during breeding and non-breeding season.

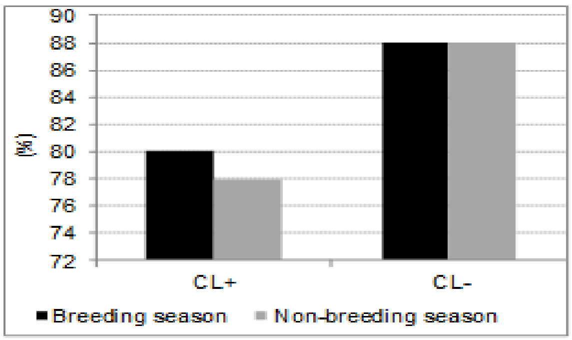

Fig. 3. Recovery rate of goat oocytes recovered from ovaries with or without CL during breeding and non-breeding season.

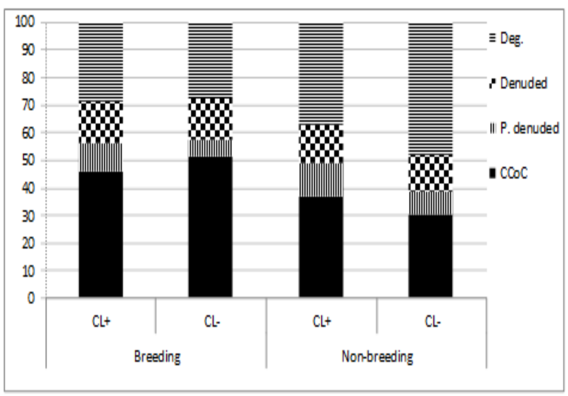

Fig. 4. Frequency distribution of goat oocytes at different categories recovered from ovaries with or without CL during breeding and non-breeding season.

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تأثير حالة المبيض وموسم التناسل على قياسات المبيض و استرداد وجودة بويضات الماعز البلدي

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تهدف هذه الدراسه التي اجريت على الماعز البلدي لمعرفة تأثير موسم التناسل و وجود الجسم الاصفرعلى المبيض على قياسات المبيض (وزن , طول, عرض وسمك), عدد الحويصلات المبيضيه , انتاج ومعدل استرادد و جودة بويضات الماعز. بعد جمع المبايض من المجازر الاهلية من برج العرب- الأسكندرية تم اخذ قياسات المبيض (وزن , طول, عرض وسمك) ، تم جمع البوبضات بإستحدام طريقة التشريح وحساب معدل الاسترداد للبويضات. لوحظ زيادة عرض المبيض (سم) بدرجة معنوية و طول , سمك (سم) و وزن المبيض(جم) 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 غياب الجسم الاصفر. كان معدل الاسترداد وانتاج البويضات أثناء موسم النشاط الجنسي عالي بدرجة غيرمعنوية وفي غياب الجسم الاصفر وذلك بالمقارنة بموسم الخمول الجنسي وفي وجود الجسم الاصفر. لوحظ ايضا متوسط عدد البويضات عالية الجودة كانت أعلى بدرجة معنوية أثناء موسم النشاط الجنسي وذلك بالمقارنة بموسم الخمول الجنسي ولم يؤدى غياب الجسم الاصفر الى زياده غير معنويه في درجات البويضات المختلفه بإستثناء البويضات المضمحله والتي زادت بدرجه معنويه في وجود الجسم الاصفر. نستخلص من هذه الدراسه تفوق انتاج وجودة البويضات فى موسم التناسل مع امكانية استرداد بويضات الماعز في غير موسم التزاوج في وجود او غياب الجسم الأصفر بدرجة مقبوله.