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Histological and Histochemical Changes of The Ovary of Goat During Autumn and Summer Seasons

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ABSTRACT:

The present study aimed to study the development and degeneration of ovarian follicles in goat during autumn and summer seasons. Seven ovaries were collected during each season from different popular markets. The ovaries were fixed in 10% neutral buffered formalin for histological examination. In autumn season; the histological examination revealed an increased ovarian activity that represented by presence of all developmental stages of ovarian follicles (Primordial follicles, primary follicles, secondary follicles, tertiary follicles and mature Graafian follicles), corpus luteum, corpus albicans and corpus heamorrhagicum. In summer season; the histological examination revealed decreased ovarian activity. The mature Graafian follicles contained degenerated oocytes and abnormal arrangement of cumulus oophorus and granulosa cells. Also, presence of persistant corpus luteum where lutein cells became small with pyknotic nuclei, and presence of large number of atretic follicles (degenerated follicles).

Key wards: *Autumn, Follicles, Goat, Histology Ovary, Summer.*

INTRODUCTION:

Goats as ruminants are multipurpose animals provide human with many products including meat, milk, and skin for leather industries, cashmere, and mohair fibers production (Gall, 1996; Simith and Sherman, 2009). Goats are seasonal polyestrous and short-day breeders. Reproduction of the goat is important to the goat keepers because they have a high fertility rate and produce many kids in a year (Jansen and Burg, 2004; Amin, 2009).

The ovaries are important part of female reproductive system that produce ova and female sex hormones, estrogen and progesterone (Erickson, 1978). Its shape may be flattened spheroids or oval to round, located near the cranial pelvic inlet (Dyce *et al*., 2010; Shively, 1984). Estrogens as steroid hormone is secreted by ovarian follicles responsible for the development of the female's secondary sex characters such as the development of the mammary gland and promote the heat signs. Progesterone a steroid hormone is secreted by the corpus luteum responsible for maintenance of pregnancy.

Various factors affecting the seasonality of reproduction include altitude, climate, breed, physiological stage, presence of the male, breeding system, and photoperiod (Fatet et al., 2011).

The present study aimed to study the development and degeneration of ovarian follicles in goat during autumn and summer seasons. Also, to study histological and histochemical structure of the goat ovary during autumn and summer seasons.

MATERIALS and METHODS:

The ovaries were collected from 14 mature female goat during autumn and summer seasons (seven samples in each season).They were collected from the different popular markets in Menoufiya Governorate, Egypt. All used animals were apparently healthy. The collected organs (right and left ovaries) were examined anatomically.

***For histological studies*:**

The samples in each season were taken immediately after slaughtering of the goat. They were fixed in 10% neutral buffered formalin solution at room temperature for at least 48 hours, then dehydrated in ascending grades of ethyl alcohol, then cleared in methyl benzoate and embedded in paraffin wax. Sections of 5-7 μm in thickness were obtained using rotary microtome **(Luna, 1968)** and stained with:
Harri's hematoxylin and Eosin (H&E).
Masson's trichrome stain.
Periodic acid Schiff stain (PAS).
Periodic acid Schiff (PAS)–orange G combination.
Alcian blue at PH 2, 5.
Periodic acid Schiff (PAS) –Alcian blue combination.

All the aforementioned stains and techniques were outlined of **Bancroft and Gamble (2002)**. The photomicrographs were taken using Leica digital camera connected with bilocular microscope.

RESULTS

I - ***Gross morphology:***

The two ovaries of goat were appeared small oval to almond in shape and pale in colour .Each ovary was located in the edge of the mesovarium near the lateral margin of the pelvic inlet on either side of the uterus within the broad ligament below the uterine (Fallopian) tubes**.** The left ovary of goat was slightly larger than the right one. Each ovary had an irregular surface by follicles of different stages of development that projected from the surface**.**

II -*Light microscopic studies:*

A –During autumn season:

The surface of the ovary of goat was covered by simple cuboidal epithelium called surface ovarian (germinal) epithelium **(Fig.1)**. The tunica albuginea was located beneath and parallel to ovarian epithelium. It consisted of dense fibrous connective tissue capsule rich in collagen fibers with few stromal cells and smooth muscle fibers. The tunica albuginea was reduced at the places, where corpora lutea and Graafian follicle approached close to it.

The parenchyma of the ovary of goat was divided into an outer cortex and an inner medulla. The cortex contained ovarian follicles and corpora lutea at different stages of development and regression. They were embedded in the stroma of the cortex. The most numerous follicles were primordial follicles that found in peripheral zone of the cortex just beneath the tunica albuginea. They were the smallest and simplest in structure. Each primordial follicle consisted of primary oocyte surrounded by a single layer of flattened (squamous) granulosa cells rest on basal lamina **(Fig.1)**.

The primary follicles were appeared larger than primordial follicles. Each primary follicle consisted of primary oocyte surrounded by single layer of cuboidal follicular epithelium. The secondary follicle consisted of primary oocyte surrounded by stratified (more than one layer of) follicular cells. Zona pellucida appeared surrounded the oocyte. The cortical stromal cells began to differentiate and surrounded the follicle forming theca folliculi **(Fig.1)**. In the tertiary follicle, the follicular cells increased in size and number as well as formation of fluid – filled cavities between them. The theca folliculi differentiated into theca interna and theca externa **(Fig.2)**.

In the mature Graafian follicle, fluid – filled cavities fused together forming one large cavity called antrum filled with follicular fluid (liquor folliculi). The follicular cells differentiated into cumulus oophorus that attached with primary oocyte, membrana granulosa that was peripheral layer rested on basal lamina, and the corona radiata that surrounded oocyte and changed to columnar cells **(Fig.3)**.The theca interna consisted mainly of epithelial cells with vesicular nuclei

and small amount of fibroblasts and connective tissue fibres. It was highly vascularized .Theca externa consisted of connective tissue ,muscle fibre layer around the follicle , less number of fibroblasts and less vascularized.

Neutral mucopolysaccharides were appeared as PAS - strong positive reaction in zona pellucida while cells of theca interna and connective tissue stroma of cortex showed moderate reaction **(Fig.4).**
Acid mucopolysaccharides were observed as alcian blue positive reaction in granulosa cells and theca externa **(Fig.4).**
After ovulation, the wall of Graafian follicle became collapsed. The antrum became smaller and the granulosa cell layer became thick and folded **(Fig.5)**. The theca externa formed connective tissue capsule from which connective tissue septa were arose carrying blood vessels and divided the corpus luteum in to lobules **(Fig .6)**. The granulosa cells became swelled and forming granulosa (large) lutein cells that were large polygonal cells with vacuolated appearance containing abundant pale eosinophilic (pink stained) cytoplasm and large spherical vesicular nuclei contained one or more prominent nucleoli. The theca interna formed theca (small) lutein cells that were small in size, with more densely stained cytoplasm and ovoid nucleus had single large nucleolus **(Fig.7)**. Fibroblasts were observed in the entire lutein tissue, among the collagen fibers and around the lutein cells. These fibroblast cells appeared small cells, ovoid or elongated, with an irregular cytoplasm and clear and large nucleus.

The cytoplasm of theca (small) lutein cells was densely packed with PAS –orange G combination positive reaction but the cytoplasm of granulosa (large) lutein cells was moderately packed with PAS –orange G combination positive reaction **(Fig.8)**.

Corpus haemorrhagicum was formed after ovulation, the follicular wall of ruptured follicle collapsed and the follicular cells were thrown into folds around a residual lumen that contained few blood clot and follicular fluid. The corpora haemorrhagica were characterized by the massive invasion of capillaries into the granulosa cells **(Fig.9)**.

The corpus albicans (whitening body) also known as atretic corpus luteum was appeared as scar tissue on the surface of ovary, formed of collagen fibers. It was stained homogenous acidophilic structure **(Fig.10).**

The medulla was consisted of loose connective tissue contained blood vessels, nerves and lymphatics. There were few of smooth muscle fibers in the connective tissue of medulla.

B – In summer season:

The structure of ovary was formed from as that appeared in autumn season **except the following: -**

Mature Graafian follicles contained degenerated oocytes and abnormal arrangement of cumulus and granulosa cells. Oocytes in these follicles were detached from cumulus oophorus and granulosa cells **(Fig.11)**.

Increase the rate of follicular atresia in ovarian cortex during summer season **(Fig.12&13)**.The atretic follicles were found in the ovarian cortex throughout the cycle. The atresia of follicles occurred in all stages of follicular development in goats. Atretic follicle was loosely connected to the stroma and there were various degrees of cellular degeneration in the granulosa and the theca interna. At later stages of atresia wall degeneration and complete follicle obliteration and connective substitution and cicatrisation was observed.

Persistence corpus luteum appeared in ovarian cortex during summer season which characterized by massive invasion of connective tissue and lutein cells became small with pyknotic nuclei

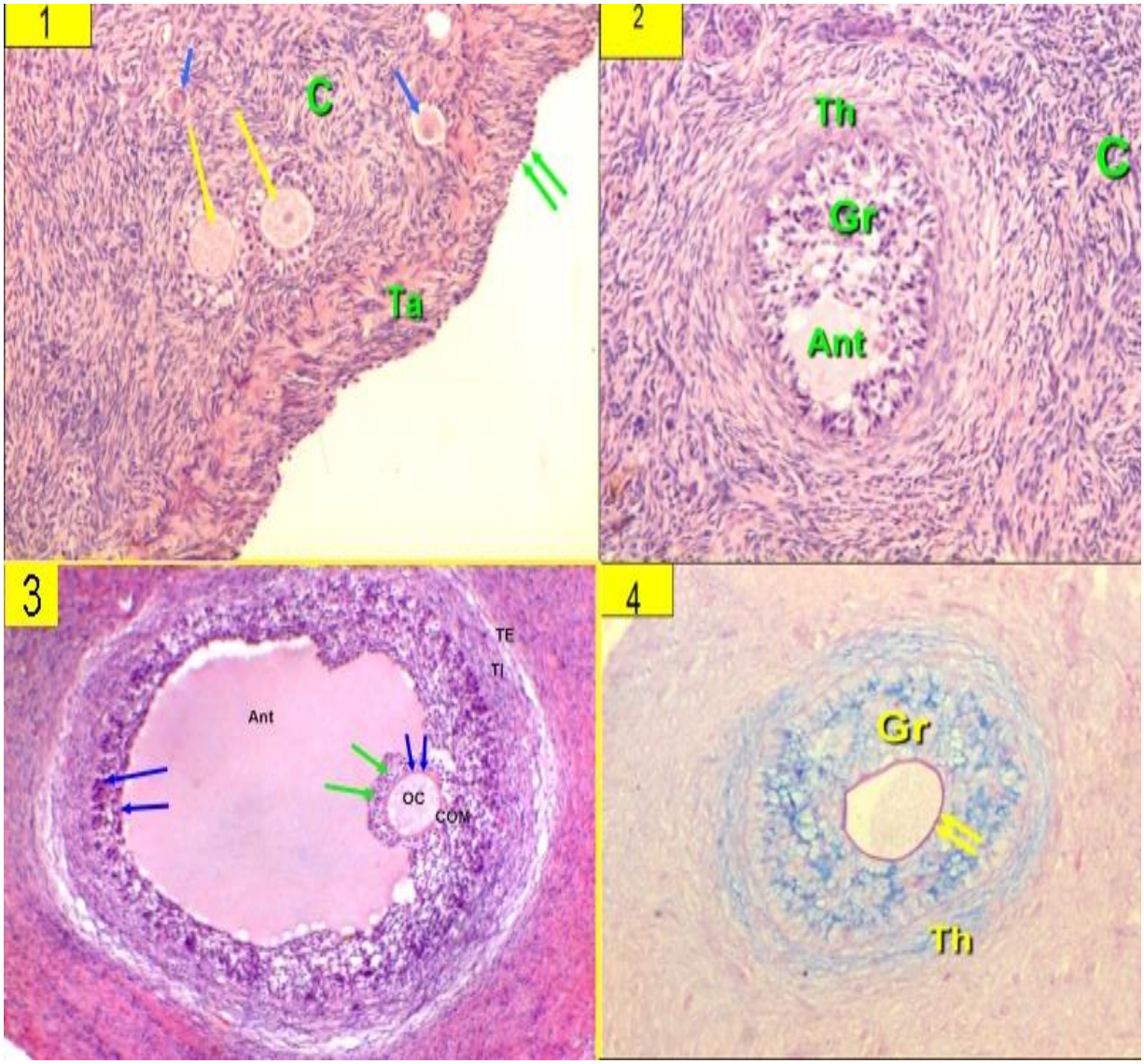

Fig.1: showing ovary of goat in autum season showing ovarian germinal epithelium (green arrows) , T.albuginea (Ta) , cortex (C) ,containing primordial follicles (blue arrows), secondary follicles (yellow arrows) (H&E X 100).**Fig.2**: showing ovary of goat in autumn season showing tertiary follicle containing , granulosa cells (Gr) , theca folliculi (Th) , antrum (Ant) , cortex (C) (H&E X 100).**Fig. 3:**showing ovary of goat in autumn season showing mature Graffian follicle containing primary oocyte (Oc),corona radiate (green arrows) ,commulus oopherus (COM) ,antrum (Ant) ,membrana granulosa (yellow arrows) , theca interna (Thi) ,theca externa (The) (H&E X 100).**Fig. 4:** showing of ovary of goat in autumn season showing secondary follicle with AB-PAS positive reaction in granulosa cells (Gr), theca folliculi (Th), zona pellucida (yellow arrows). (AB pH 2.5 – PAS stain X 100).

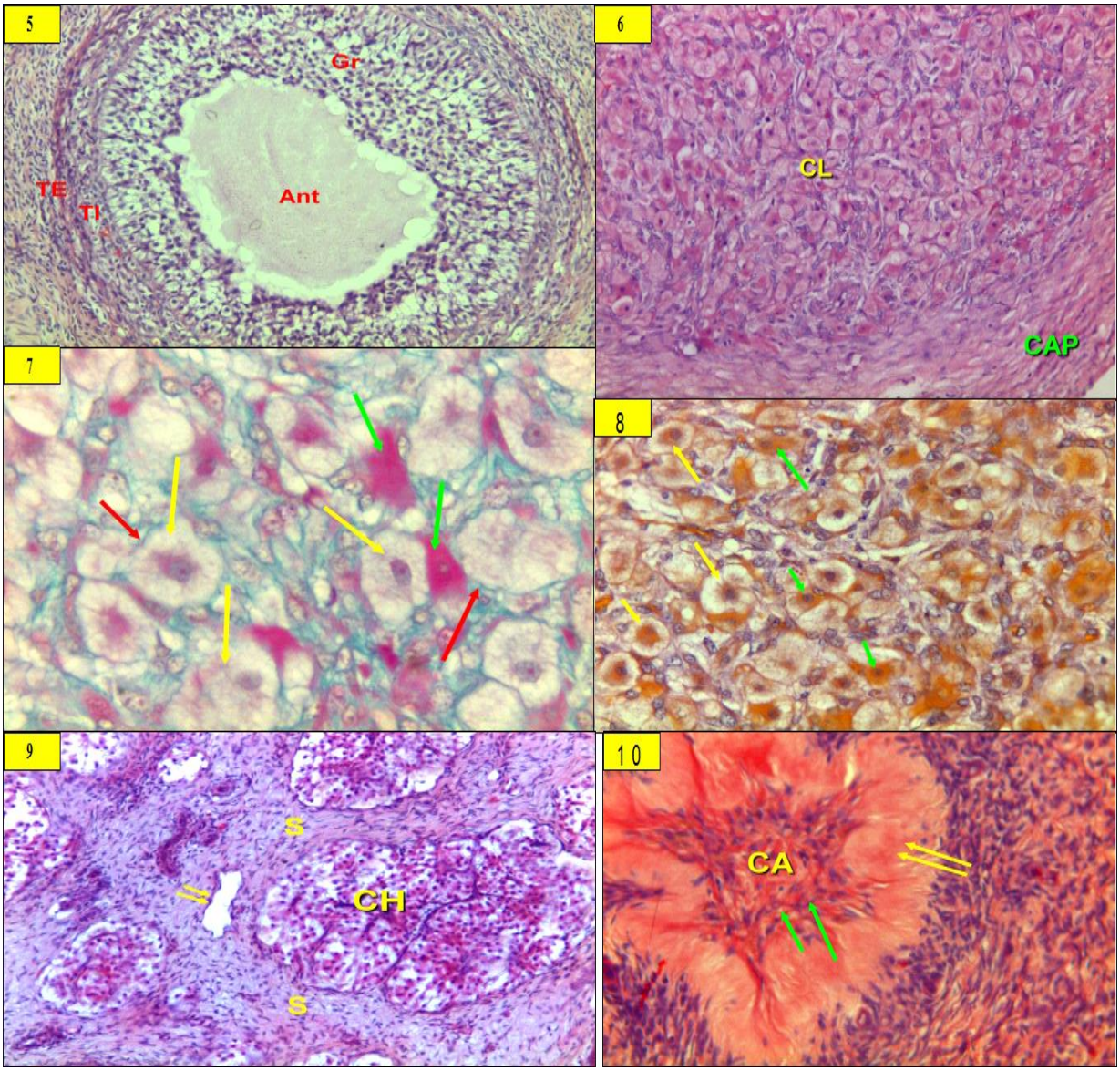

Fig.5: showing mature Graafian follicle in autumn season after ovulation and release oocyte, folding and separation of granulosa cells(Gr), theca interna(TI), theca externa(TE), antrum (Ant).(H&E X 100).**Fig.6:** showing ovary of goat in autum season showing connective tissue capsule (Cap), corpus luteum (CL) .(H&E X 100). **Fig.7:** showing distribution of collagen fibers in corpus luteum around lutien cells (red arrows), granulose lutein cells (yellow arrows), and theca lutein cells (green arrows) in autum season. (Masson trichrome stain X 400).**Fig.8:** In autum season showing theca lutein cells showed strong positive reaction (green arrows) ,granulosa lutein cells showed positive reaction in center around nucleus and negative reaction at periphary (yellow arrow) . (PAS –OG combination X200).**Fig. 9:**showing corpus haemorragicum (CH)in autumn season, connective tissue septa (S) , blood vessel(yellow arrows) .(H&E X100).**Fig.10:** showing corpus albicans (CA),macrophage and fibroblast for luteolysis (green arrows),fibrous c.t (yellow arrow) . (H&E X400

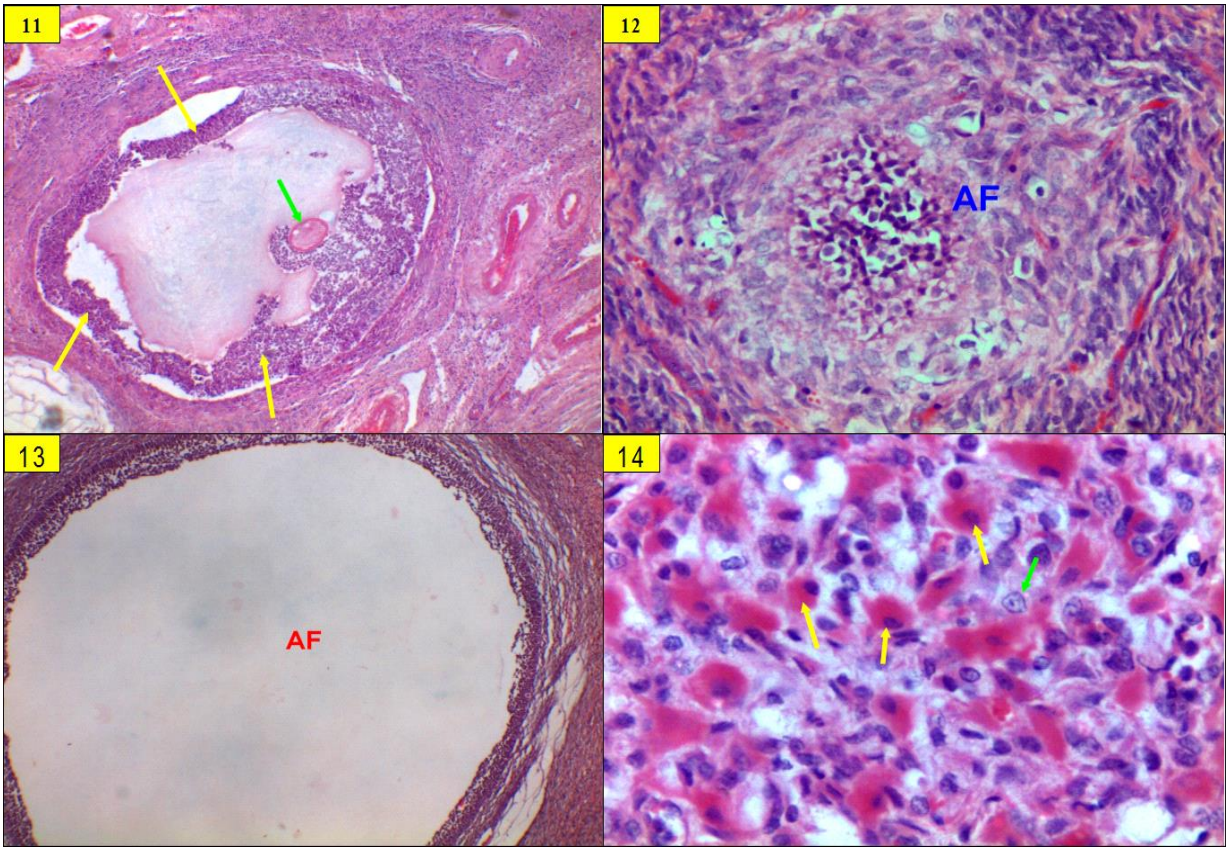

Fig.11: A photomicrograph of ovary of goat during summer season showing mature Graffian follicle containing degenerated oocyte (yellow arrows) abnormal arrangement of granulosa cells (green arrow). (H&E X40).**Fig.12&13:** A photomicrograph of ovary of goat during summer season showing atretic follicle (AF) . (H&E X200).**Fig.14:** A photomicrograph of ovary of goat in summer season showing persistant corpus luteum, lutein cells became small and pyknotic N. (H&E X400)

DISCUSSION:

The present study revealed that the ovarian activity during autumn season, affirmed by all developmental stages of ovarian follicles (primordial follicles, primary follicles, secondary follicles, tertiary follicles and mature Graafian follicles, corpus luteum and corpus albicans and corpus haemorrhagicum) and most of follicles were found healthy.

On the other, ovarian inactivity during summer season was obviously noticed in the form of mature Graafian follicles containing degenerated oocyte with abnormal arrangement of granulosa cells, presence of persistence corpus luteum and lutein cells became small with pyknotic nuclei. The same results were recorded by Banks et al., (1983). They concluded that this ovarian inactivity due to after parturition serum P4 decreases to the baseline level, although the structure of corpora lutea might be conserved by the luteotrophic action of prolactin.

The presence of large number of atretic follicles during summer season. The atresia occurred due to insufficient gonadotrophins secretion Guraya (1979).

The present investigation showed lower rate of follicular development during summer season than autumn season; this agree with the findings of Bari et al.,(2011) and Wolfenson et al., (1995) who reported that heat stress decreased follicular development.. High temperatures had negative effect on reproductive function (Putney et al., 1989; Edwards and Hansen 1997; Lawrence et al., 2004).

In the same respect, Arthur et al., (1995); Abdel Rahim and El Nazier (1992) in camel, reported that the incidence of inactive ovaries reached its peak during summer.

In Saudi Arabia Arthur et al., (1995) reported that breeding occurs throughout the year under good nutritional condition in camel. This is in contrast to the observations of Akral and Khanna (1995), Amer (2004), Sarhan (2007), Zeidan *et al.,* (2008) and El-Harairy et al., (2010) who reported that dromedary she–camel's ovary showed higher activity in spring and winter than summer and autumn seasons.

This result in contrast to the observations of Shah et al., (1991) who reported that the ovarian activity in buffaloes increased during the winter, decreased in autumn and spring, and was lowest in the summer. But the Zebu cattle, breeding activity increased during the summer.

This result in contrast to the observations of Elhassan and Tingari (2015) in she-camel, who observed that hemorrhagic follicles (anovulatory follicles) observed in the ovarian cortex during autumn season with increased number.

CONCLUSION:

The present study revealed that follicular development is increased in autumn season than summer. In summer season heat stress decrease follicular development, which cause increased degenerated ova and effect on reproductive function.

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