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PREDICTION FOR THE TRANSITIONAL PERIOD AND INCIDENCE OF BREEDING SEASON BASED ON ABATTOIR EWES OVARIAN SAMPLES

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ABSTRACT
The main objective purpose of this study is to create a simplest and cheapest predictive source for the incidence of the transitional period toward the breeding season of ewes in regarding to the follicular developmental degree which related to the ovarian follicular waves at the quiescent period to be prepared for ewe's breeding season. Ewes genitalia (2-3 year old) were collected directly after slaughtering at Al-Shoáalla abattoir west-north the capital (Baghdad) early morning, preserved at refrigerated containers (4-8°C), transferred directly to the theriogenology Lab. from April to June 2017, divided into four groups of 20 ovaries (4X20). Ovaries were separated from all surrounded tissue washed thoroughly with dis. water then with normal saline contain antibiotics with antifungal. Oocytes collected by two ways aspiration by plastic syringes connected to 18 gauge needles, and slicing methods, collected oocytes were evaluated and counted then followed the process of maturation and IVF. Ovarian size for the three months (April, May and June) collected samples reveal (mean) 4.47mm, 5.81mm and 8.37 mm respectively. Ovarian weights were 0.72, 1.25 and 1.75gm for the three collected samples respectively. Follicular development and CL bearing ovaries were more presented with samples of late May and June. Collected oocytes were done by two methods (Aspiration and Slicing) and the total oocytes number for three months respectively was 192, 240 and 353 ova. Oocytes quality was also demonstrated by three parameters (Good, fair and poor) the index be 53.3%, 41.3% and 5.3% for the three months by Aspiration method and by Slicing method was 36.47%, 37.88% and 25.63% for the three months respectively. It is so important to predict the time of transitional period for ewes breeding season in related to the changing in ovarian parameters using ovarian abattoir samples which the cheapest, more practical and easily handling.

KEYWORDS: follicular developmental, Al-Shoáalla abattoir, Aspiration method, ewes breeding season.

INTRODUCTION

Reproduction in non-human mammals is regulated by a physiological event known the estrus cycle, in which, an alteration in hormonal surge modulates the ovarian activity and sexual responses makes females more receptive to males of own species, many factors influence this event accompanied the hormonal changes; photoperiodism is one of more important factors that makes this event (estrus cycle) under controlling of season as in sheep[1]. In tropical regions, near the Equator, where there is little variation in the length of day light periods, there is a tendency for seasonal breeders to present reproductive activity along the year[2, 3], while in Subtropical area as in our land (Iraq) this effect will be variable. Seasonality of species like sheep which is a short daylight breeder is started from autumn and ends in winter, with anestrum period at spring and summer, sometime those breed in the Mediterranean have a longer breeding season[1]. Breeding season of sheep starts in the early autumn when days-light period being shorter, and ends in mid-winter when day length is increased again. Ovulation then ceases, and the animal remains anovulatory during the long days of spring-summer[4]. Some breeds of sheep are highly seasonal in terms of reproductive capability; these changes are regulated by photoperiod and melatonin secretion[5]. There are many other factors that change length of photoperiod like fluctuations in environmental temperature, climate variation, availability and quality of feed. The changing photoperiod acts as a bio-regulator of reproductive activity and fertility through the mediation of central nervous system, hypothalamus, adenohypophysis and the pineal gland. Onset of breeding season in seasonal breeds is much similar to the onset of puberty[6]. Transition from non-breeding (anestrous) to breeding (estrous) represents sexually quiescent state to active state[7]. Non breeding season is characterized by an increase in negative feedback effect of estrogen on GnRH and gonadotrophin secretion as is the case in pre-pubertal period, this results in reduced frequency of GnRH pulses, suppressing the gonadotrophin drive to the gonads thereby causing the gonadal regression and toward puberty the hypothalamo gonadal axis is under the negative feedback effect of the estrogen, the pulse frequency of GnRH/LH is insufficient to stimulate the development of follicles[8,9]. Sustained increase in estrogen activates the surge center of the hypothalamus thereby creating a positive feedback effect on the hypothalamus causing surge release of GnRH/LH. Pre puberty, the small content of estrogen from the developing follicle exerts a negative feedback effect on the hypothalamus, while at puberty the consistently high estrogen exerts a positive feedback effect there by activating the surge center of the hypothalamus and causing for the surge release of LH which causes for the maturation of the follicle[10] Pre pubertal period is comparable to non-breeding or anestrous period while the puberty is comparable to the onset of breeding season.

Similar kind of transition occurs in both the cases[11]. Therefore the primary mechanism in seasonal breeding is the neural control of pattern of GnRH from hypothalamus. Pulsatile secretion of GnRH in turn increases the LH and FSH from the pituitary thereby activating the gonads, the seasonal change in sensitivity to estrogen is the major mechanism for shift from breeding to non-breeding season [12]. The objective of this study is designed to investigate the possibility to predict the incidence of ewe breeding season transitional period in depending upon the ovarian changes of abattoir[13] samples includes its effect on ovarian weight, size, ovarian structures (follicular development and Corpus luteum present) ova collection methods ova number and ova quality[14].

MATERIALS & METHODS

Ethical approval

An ethical approval was not necessary since no live animals were used for this study; however, the samples were collected from alshaálla abattoir North-West Baghdad the capital.

Location of the study

This study was conducted at the department of Surgery and Obstetric Lab. at College of Veterinary Medicine, Baghdad University at Al-Jadria Location on period between April 2017 to June 2017, at that time ambient or local temperature is ranged between 25-45°C, in which, the study supposed to be done in period out of ewe breeding season in which it starts after that time.

Samples collection

Ewes (2-3 years old) genitalia were collected directly from Al-Shoáalla abattoir north-west of Baghdad, transported by cooled container to the laboratory at the collage of Vet. Medicine \ University of Baghdad from April 2011 to June 2017 [15]. Ovarian samples were prepared as mentioned by [16], firmly and smoothly separated from surrounded tissues by clean scissor washed more than one time with dis. water then with normal saline, kept in becker containing MEM with antibiotic solution for settlement at room temperature (RT) for 5-10 minutes and prepared for further processing. Ten genitalia samples were collected each time weekly, in which, twenty ovaries were collected per one time every one week (Fig.1, 2, 3).

FIGURE 1: Ewe genitalia with an arrow denoted the ovary

FIGURE 2: Ewe genitalia with an arrow denoted ovary bearing small follicle

FIGURE 3: Collected ovaries after separation and washing

Samples processing

Weekly collected ovarian samples (20 ovaries) were divided to four groups, five ovaries each, in which, four groups for each months, ovarian weight and size were measured and recorded. Oocytes were collected for each group by two methods the aspiration and slicing, collected oocytes were counted and evaluated as mentioned by[17] all results were recorded.

FIGURE 4: ovarian size mesearment

FIGURE 5: Aspiration method for oocytes collection

FIGURE 6: Slicing method for oocytes collection

RESULTS

All ovarian samples were conducted to weight and size measurement and the final result taken as the mean of the one group in related to three months

Effect of the three months period upon ovarian size

The effect of the three months on ovarian size of the four collected group declared that there is a transitional period appeared by increasing ovarian size toward the breeding season is mentioned in table (1), in which, transitional period significantly ($P<0.05$) affect the ovarian size.

TABLE 1: Effect of three month period on the ovarian size (mm)

Month	Ovarian size mm				
	Group 1	Group 2	Group 3	Group 4	Total
April	4.062	4.34	4.78	4.70	4.47
May	5.74	5.94	5.68	5.86	5.81
June	7.78	8.14	8.65	9.18	8.37
Total	5.86	6.14	6.37	6.58	

Effect of the three months period upon ovarian weight

Results showed that ovarian weight increased progressively as time passed from April toward June, mainly this progressive weight increasing referred to the transitional phase adjust the breeding season as shown in tab.(2)

TABLE 2: effect of transitional period on the ovarian weight, ovary appeared to be progressively gain weight due to elevated number of developed Oocytes toward the Breeding season.

Month	Ovarian weight gm				
	Group 1	Group 2	Group 3	Group 4	Total
April	0.602	0.782	0.748	0.758	0.72
May	1.04	1.24	1.29	1.41	1.25
June	1.63	1.60	1.83	1.92	1.75
Total	1.1	1.2	1.29	1.36	

Follicular development

Ovarian samples of April period showed small and scattered follicles, this was more developed at the next month but still with no CL or no ovulation occurred, while

some ovarian samples of June (almost) contained large follicle with or without CL, this indication related to the incidence of the ovulation(might be silent), figure (7, 8, 9, 10).

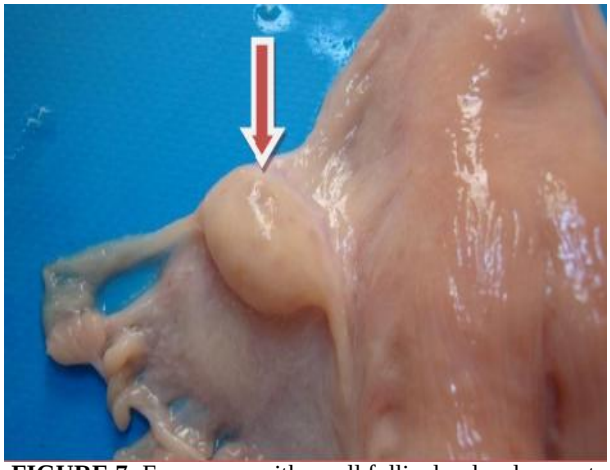

FIGURE 7: Ewe ovary with small follicular development with no CL

FIGURE 8: Ovarian sample with moderate follicular development

Figure 9: Ewe genitalia with two ovaries left one bear two corpola lutea refered to double ovulations

FIGURE 10: Ovarian sample with well developed follicle

Effect of transitional period upon Oocytes collection

Oocytes collection was applied by two methods, aspiration and slicing the ovarian samples by surgical blade. Aspiration method for oocytes collection yield no or fewer Oocytes mainly through April and May months and little number through June period in which follicular development still not so active to yield more Oocytes. Slicing of ovarian samples yield less to moderate Oocytes number at April and May to be increased in number through June period which an indication that the ovarian follicular development was start to be activated while ewes entered the transitional period toward the breeding season (Table 3).

TABLE 3: Effect of transitional period on Oocytes collection

Month	Aspiration				Slicing				
	Group1	Group 2	Group 3	Group 4	Group 1	Group 2	Group 3	Group 4	Total
April	X	X	2	4	35	43	52	56	192
May	4	6	6	9	43	51	58	63	240
June	8	12	12	12	66	73	83	87	353
Total	12	18	20	25	144	167	193	206	785

Collection of Oocytes by aspiration method yield less Oocytes number, while slicing method yield more, result will be changed as ewes enter the transitional period toward the breeding season

Effect of the transitional period on the collected oocytes quality

Result showed that; as time proceed oocytes quality changed and improved, in which number of good quality Oocytes increased as mentioned in tab.(4). Oocytes quality in regarding to the cumulus cells, cytoplasm (Fig. 11, 12, 13).

TABLE 4: effect of transitional period on oocytes quality

M		April		May				June						Total	%
		G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4	G_1	G_2	G_3	G_4		
Aspi.	G	x	x	1	1	2	2	3	4	4	7	8	8	40	53.3
	F	x	x	1	3	2	3	3	3	3	5	4	4	31	41.3
	P	x	x	x	x	x	1	x	2	1	x	x	x	4	5.3
Slic.	G	10	12	14	15	13	15	19	22	30	32	37	40	259	36.47
	F	13	15	20	22	18	21	22	24	21	29	33	31	269	37.88
	P	12	16	18	19	12	15	17	17	15	12	13	16	182	25.63
Total		35	43	54	60	47	57	64	72	74	85	95	99	785	

M= method, Aspi. = Aspiration method of Oocytes collection, Slic= Slicing method of Oocytes collection, $G_?$ = group of ovarian sample, G= good quality oocyte, F= fair quality oocyte, P= poor quality oocyte.

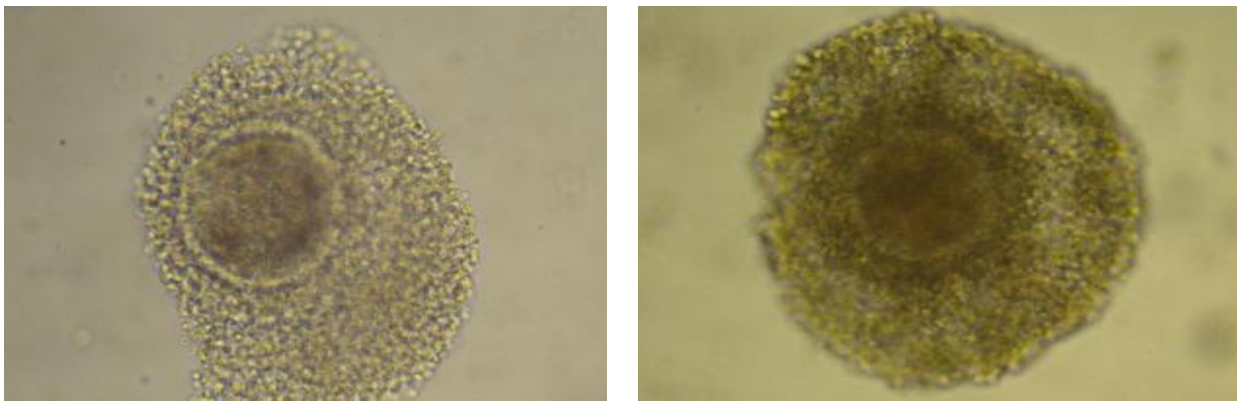

FIGURE 11: Good quality oocyte

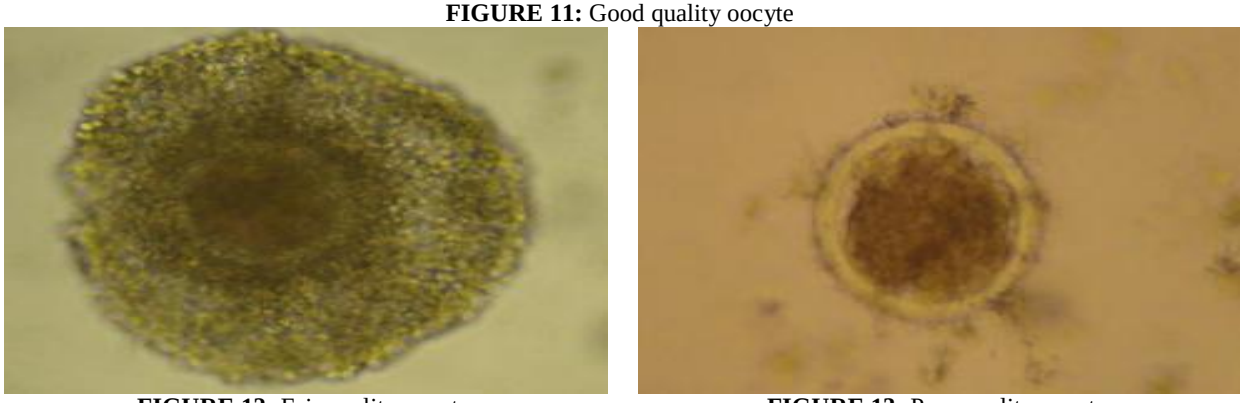

FIGURE 12: Fair quality oocyte

FIGURE 13: Poor quality oocyte

DISCUSSION

Most mammals living at temperate latitudes exhibit marked seasonal variations in reproduction. In long-lived species, it is assumed that timely physiological alternations between a breeding season and a period of sexual rest depend upon the ability of day length (photoperiod) to synchronize an endogenous timing mechanism called the circannual clock. Sheep has been extensively used to characterize the time-measurement mechanisms of seasonal reproduction. Melatonin, secreted only during the night, acts as the endocrine transducer of the photoperiodic message[18]. This study was design to predict the transitional period of ewe cyclicity development from anestrum period or time of excessive (prolonged) daylight toward breeding season in regarding to the morphological, structural and oocytes collection (number and quality) occurred in the ovaries of abattoir ewe genitalia samples which is the most cheapest, accurate and easy manipulated method. The changing photoperiod acts as a bioregulator of reproductive activity and fertility in sheep through the mediation of central nervous system, hypothalamus, adenohypophysis and the pineal gland. Onset of breeding season in sheep which is a typical seasonal breeder is much similar to the onset of puberty. Transition from non-breeding (anestrous) to breeding (estrous) represents sexually quiescent state to active state [19,20]. These physiological changes directly affect the ovarian morphology by increasing follicular number and size then weight and size of the entire organ (the ovary) will increase, these results agreed with[21] in which; in non

breeding season there is an increase in negative feedback effect of estrogen on GnRH and gonadotrophin secretion and this results in reduced frequency of GnRH pulses, suppressing the gonadotrophin drive to the gonads thereby causing the gonadal regression[9].

Results of this study found that; there is a continuous ovarian weight and size elevation as time passed from April, May and toward June and these findings are quiet similar to changing from Pre-pubertal period (comparable to non-breeding or anestrous period) to puberty (comparable to the onset of breeding season), and these finding agreed with [23] in which; similar kind of transition occurs in both examples. Therefore the primary mechanism in seasonal breeding is the neural control of pattern of GnRH from hypothalamus and pulsatile secretion of GnRH in turn increases the LH and FSH from the pituitary thereby activating the gonads which demonstrated by increasing weight and size [24].

The increasing in follicular number and size which start mainly with the ovarian samples of April- group 3 toward the other months-groups consecutively, these follicular changes may be due to the changing in the hormonal status of the donor ewes, this transitional period also observed by [25], in which he was concluded that; the growth of ovarian antral follicles to an ovulatory size was maintained throughout anoestrus in ewes, with a transient shift in the number of small and medium-sized follicles during mid-anoestrus, and that the periodic emergence of waves of large follicles (>or =5 mm in diameter) occurred in synchrony with an endogenous rhythm of FSH secretion. These results were in agreement with[22] mainly those related to the ovarian samples which were bearing Corpora lutea collected on May toward June, the ovulation rate might be started by this period in connection with FSH level changing under influence of other factors as daylight, Melatonin release and so on.

The effect of transitional period upon oocytes collecting method

Aspiration method for oocytes collection still yield less oocytes count with good quality, result of this study found that this method is more practical and more effective to perform when the ovary with moderate to large follicles (>5mm diameter) and not effective with follicles less in diameter (< 2mm diameter), this is observed by [26] that he conducted this method mainly when the oocytes collection are tried within season and mainly for large size follicle. [16] Observed the same results in oocytes collection from abattoir samples, that the aspiration method is easily conducted within breeding season as found by this study that aspiration yield moderated Oocytes count in regarding to slicing one.

Effect of transitional period upon oocytes quality

The result showed that; there is an oocyte quality improvement appeared as an increasing in the number of good quality oocytes in regarding to the other two grades (fair and poor quality), and this quality improvement may be due to the effect of a circadian rhythm of day light toward the transitional period, and to the fact that; the changing in daylight duration will be at optimal limit on middle to the end of May then it goes down gradually to be equal at mid August (breeding season). This is agreed with[28] in which the season affects yield and quality of blastocyst in the way that the autumn period is more favorable for embryo development, and this is mainly initiated from the good quality of oocytes collected through the period of decreased daylight (autumn)[29]. agreed with the results found in this study, the effect of daylight might influence the cyclicity of ewe which affect ovarian functions, modulate hormones action by increased its level and then more follicular development as this period proceed and changed from transitional to breeding season.

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