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The Affect of Breeding Season of Iraqi Sheep in Relation to Number, Quality and Maturity Index of abattoir Ovarian Oocytes

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Abstract:

This study is designed to investigate the transitional period of sheep breeding season under Iraqi environment which is mainly hot and the effect of this hot weather on sheep fecundity in relation to its abattoir ovarian samples and oocytes quality. 48 ewe genitalia were collected directly after slaughtering, 24 genitalia on November 2018 (within season), and 24 genitalia at March 2019 (out of season), six specimens each. After separation from entire genitalia, ovaries washed thoroughly with dis. water then with normal saline contain antibiotics with antifungal, ovarian weight and size will be calculated for both season. Oocytes collected by ovarian slicing method, evaluated and counted, then incubated in CO2 incubator for 30 hours for maturation. Collected ovarian size (means) 4.062 ±0.02mm, 4.34±0.33mm, 4.78±0.43 mm and 4.47±0.38mm (out of season), 7.78±0.23mm, 8.14±0.33mm, 8.65±0.45mm and 9.18±0.48 mm within season. Ovarian weights were 0.602±0.24g, 0.782±0.40g, 0.748±0.57g and 0.72±0.56g out of season, 1.634±0.44g, 1.686±0.56g, 1.830±0.23g and 1.750±0.28g. Oocytes number are 28 (22.04%), 34(26.77%), 30(23.62), 35(27.55%) for out of season time, and 66(22.14%), 73(24.50%), 76(25.50%) and 83(27.85%) within season time. Oocytes evaluation were 8(28.57%), 10(29.41%), 6(20%), 7(20%) good quality, 10(35.71%), 13(38.23%), 10(33.33%)and 11(31.42%) fair oocytes, while the poor quality were 10(35.71%), 11(32.35%), 14(46.66%) and 17(48.57%) out of season and 33(50%), 38(52.05%), 35(46.05%) and 44(53.01%) good quality, 22(33.33%), 25(34.24%), 20(26.31%) and 25(30.12%) fair quality oocytes and the poor quality are 11(16.66%), 10(13.69%), 21(27.63%) and 14(16.86%) within season. In conclusion, sheep breeding season under Iraqi ecological factor in relation to the ovarian activities started from end of August to the end of February with a direct effect of season on Iraqi sheep sexuality.

Key words: sheep, oocyte, ovary, season, follicle, genitalia

Introduction:

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Seasonal breeders are animal species that successfully mate only during certain times of the year. These times of year allow for the optimization of survival of young due to factors such as ambient temperature, food and water availability, and changes in the predation behaviors of other species.Related sexual interest and behaviors are expressed and accepted only during this period, female seasonal breeders will have one or more estrus cycles only when she is "in season" or fertile and receptive to mating **(Prendergast , 2005)** The hypothalamus is considered to be the central control for reproduction due to its role in hormone regulation(**Lehman et al ., 1997**). This is achieved specifically through changes in the production of the hormone GnRH, which in turn transits to the pituitary where it promotes the secretion of the gonadotropins LH and FSH, both pituitary hormones critical for reproductive function and behavior, into the bloodstream, changes in gonadotropin secretion initiate the end of anestrus in females **(Williams et al .,2017)**.Photoperiod likely affects the seasonal breeder through changes in melatonin secretion by the pineal gland that ultimately alter GnRH release by the hypothalamus, seasonal breeders can be divided into two groups based on fertility period, "Long day" breeders cycle when days get longer (spring) and are in anestrus in fall and winter as mare, and "Short day" breeder's cycle when the length of daylight shortens (fall) and are in anestrus in spring and summer as ewe. The decreased light during the fall decreases the firing of the retinal nerves, in turn decreasing the excitation of the superior cervical ganglion, which then decreases the inhibition of the pineal gland, finally resulting in an increase in melatonin. This increase in melatonin results in an increase in GnRH and subsequently an increase in the hormones LH and FSH, which stimulate cyclicity **(Senger ,2005).**Melatonin is a modified amino acid secreted by the pineal gland that communicates information about environmental lighting to various parts of the body. Melatonin has important effects on maintaining biological rhythms, and has important effects on reproductive function. Light that is exposed to the retina is first relayed to the suprachiasmatic nucleus of the hypothalamus, an area of the brain well known for coordinating biological clock signals Fibers from the hypothalamus descend to the spinal cord and project back to the pineal gland. Thus it converts signals from the sympathetic nervous system into hormonal signals **(Bowen,2009).**Estrous cycles are commonly affected by the seasons in different species; ovine is one of those which are very receptive. It is due to the number of hours daily that light enters the eye of the animal. It then affects the brain, to determine the release of certain precursors and hormones. Most sheep are seasonally polyestrus short-day breeders. They start their estrus when the length of the day begins to decrease; estrous cycles will continue every 16 to 17 days until the ewe is bred or returns to anestrus **(Schoenian, 2009).** Therefore most breeding in ovine occurs in the months between October and November. Estrus phase approximately 24 to 36 hours, and is where the ewe will stand to be mounted by the male. Detecting estrus in sheep is much harder than that is cattle especially dairy cattle; it is almost required to have a ram present(**Schoenian, 2009)**) . On conclusion of (**Panel** et al**.,2008**) that; oocytes collection in regarding to the best quality and elevated number can be successfully incorporated in ewes through breeding season (fall), a higher proportion of good quality embryos with high freezability index being obtained in the fall.

Materials and methods:

This experiment is well designed to conduct on abattoir ewe genitalia specimens that already obtained and collected directly after slaughtered.48 ewe genitalia were collected (Two

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attempts 24each) directly after slaughtering at Alshaálla abattoir (North-west) from Baghdad, first (24genitalia) on November 2018 (within season), second attempt (24 genitalia) on March 2019 (out of season). All genitalia specimens were transferred by cool box from sight of collection (abattoir) to the Surgery and Theriogenology department at the college of veterinary medicine \ Alamirreia University of Baghdad. Specimens collection, manipulations, transferring then oocytes retrieved were preceded as discussed by (**Saleh, 2016).**)· Out of season collected specimens were characterized by its small size genitalia, ovaries contain scattered small size follicles with no corpus luteum (CL). While those genitalia which were collected within season appeared as well developed system, large size ovaries were contained either large follicles or well developed corpora lutea.

Ewe genitalia were collected out of season

One of the out season collected ewe genital system, orange arrows denoted small non-functional ovaries

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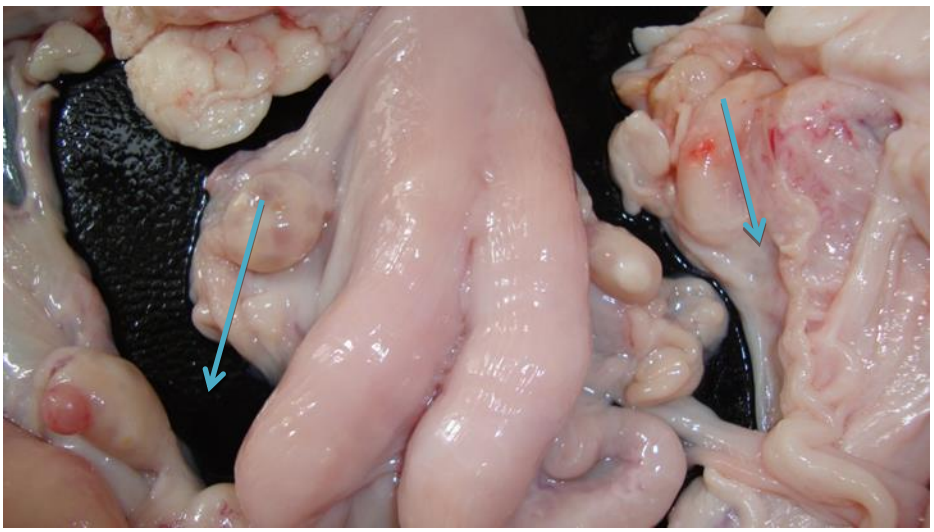
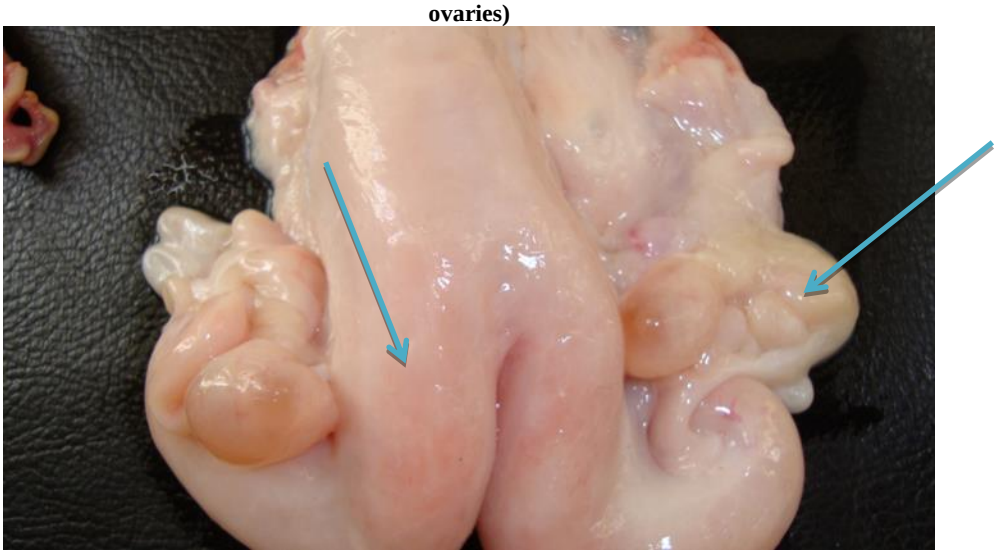


Ewe genitalia were collected within season; blue two arrows denoted corpora lutea (functional ovaries)



One of within season collected ewe genital system; two blue arrows denoted follicles (functional ovaries)

Separation of the ovaries (both seasons) from entire genitalia specimens, kept in small glass container (becker), washed thoroughly with warm water then distilled water, keep it in water bath (37°C) for 30-60 minutes for further settlement. Ewe genitalia divided for four groups, each groups of six genitalia, that is mean 12 ovaries. Ovaries weight and size were calculated, then moved to glass Petri dish, slicing to small pieces, the field then examined by inverted microscope for oocytes retrieval **(Wani et al.,2013).** All results were recorded for further analysis; collected oocytes were cultured in CO2 incubator for 28-30 hrs, and then evaluated.

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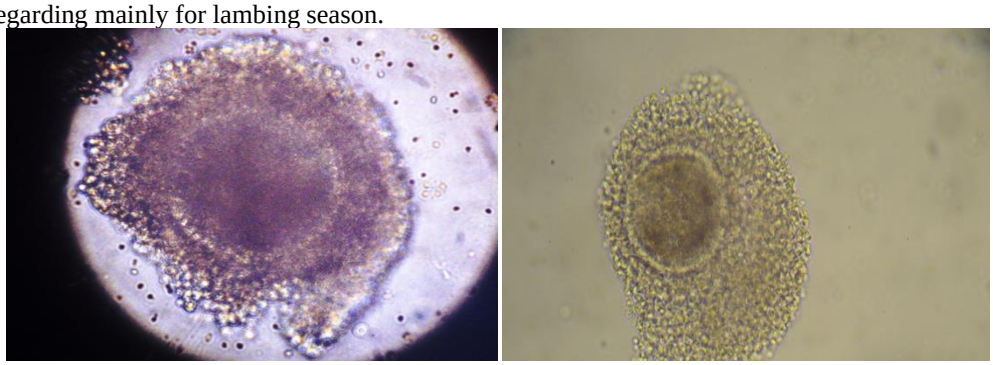
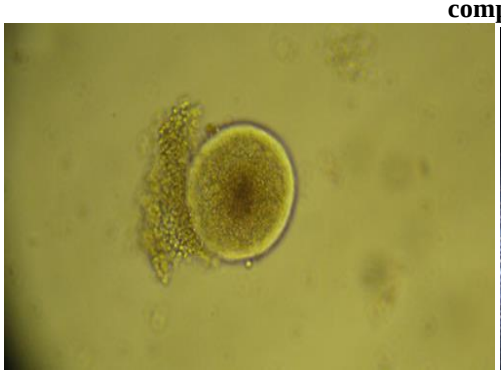
Result:

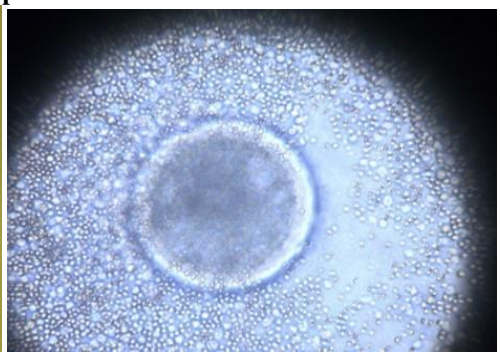
The effect of three seasons winter, summer and rainy on the recovery and quality of sheep oocytes were studied well by (**Shivam and Anil 2018).** Among the three seasons, the winter season appeared superior (P<0.05) in terms of recovery of good, fair, total and usable (Good+Fair) oocytes and number of poor quality oocytes in summer and rainy season were significantly higher (P<0.05) compared to winter. The results showed winter season is better than summer and rainy season to recover a greater number of good as well as fair quality oocytes from sheep ovaries, winter season is sheep breeding season while rainy season is regarding mainly for lambing season.



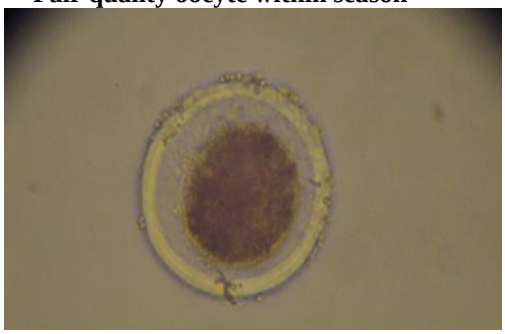
Good quality within breeding season (November) oocytes surrounded by cumulus cell complex



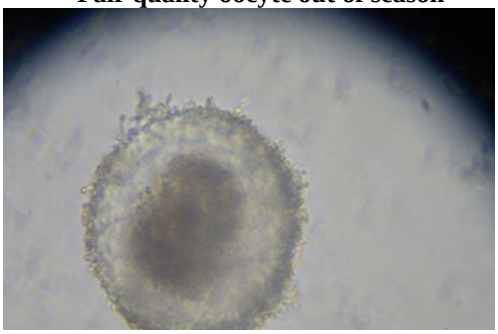
Fair quality oocyte within season



Fair quality oocyte out of season



Poor quality oocyte within season



Poor quality oocyte out of season

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All ovarian samples were conducted to weight and size measurement (both season), to evaluate the effect of season on functionality.

1-Effect of season upon ovarian weight and size

The effect of the season upon ovarian samples weight and size were calculated in which; all samples were divided into four aliquots, each aliquot involved six ovaries for both within and out of season, and this design was conducted to count these parameters correctly. Seasonal effect was significantly ($P<0.05$) affect the ovarian size and weight, and this was mainly due to the increase in functional sexual activates via ewe breeding season .

Tab.(1) Effect of season upon ovarian weight (gm) and size (mm).

Parameter	November (Within season groups) mean ±				March (Out of season groups) mean ±			
	G*.1	G. 2	G.3	G.4	G.1	G.2	G.3	G.4
Weight (gm)	1.634 ± 0.44	1.686 ± 0.56	1.830 ±0.23	1.750 ± 0.28	0.602 ± 0.24	0.782 ± 0.40	0.748 ± 0.57	0.72 ± 0.56
Size (mm)	7.78 ± 0.23	8.14 ± 0.33	8.65 ± 0.45	9.18 ± 0.48	4.062 ± 0.02	4.34 ± 0.33	4.78 ± 0.43	4.47 ± 0.38

G=group

2- Effect of season upon ovarian oocytes collection

Results shown that; there was a seasonal direct effect on oocytes account between within and out of breeding season (tab.2), samples collected within season yield more oocytes count than those collected out of season.

Tab.2: Effect of season on oocytes number

Season	Ovarian group								Total	
	G 1	%	G2	%	G3	%	G4	%	Oocyte	%
Within season	66	22.14	73	24.49	76	25.50	83	27.85	298	70.11
Out of season	28	22.04	34	26.77	30	23.62	35	27.55	127	29.88
Total	94		107		106		118		425	

●The oocytes account percentage between groups was related to the oocytes number per total oocytes account.●●The total percentage was related to the seasonal oocytes account per total oocytes number.

3- Effect of season on oocytes quality

a- Within season

Result shown that; during breeding season oocytes quality of good grade was markedly increased and this was mainly due to increase ovarian activities and functionality (tab.3)

Tab. 3 (a) Effect of seasonality on ovarian function (within season)

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Oocyte grade	Group1			Group2			Group3			Group 4		
	No	M.	% Group 2 %% %	No	M	%	No	M	%	No	M	%
Good	66	33	50	73	38	52.05	76	35	46.05	83	44	53.01
Fair	66	22	33.33	73	25	34.24	76	20	26.31	83	25	30.12
Poor	66	11	16.66	73	10	13.69	76	21	27.63	83	14	16.86

- **No= Number of oocytes, ●●M= oocyte maturation,●●●% maturation per total oocytes number**

b- Out of season: Results showed that;

Tab. 3 (b) Effect of seasonality on ovarian function (out of season).

Opposite to incidence of breeding season, ovarian samples that collected out of season yield less oocyte with low quality in which fair and poor grade showed high percentage (tab3).

Oocyte grade	Group1			Group2			Group3			Group 4		
	No	M.	% Group 2 %% %	No	M	%	No	M	%	No	M	%
Good	28	8	28.57	34	10	29.41	30	6	20	35	7	20
Fair	28	10	35.71	34	13	38.23	30	10	33.33	35	11	31.42
Poor	28	10	35.71	34	11	32.35	30	14	46.66	35	17	48.57

- **No= Number of oocytes, ●●M= oocyte maturation,●●●% maturation per total oocytes number**

Results showed that;

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1-abattoir genitalia specimens comprised as a very good sources for gametes utilization if tolerated well.

2-Under Iraqi conditions, sheep which is a seasonal breeder exhibited a tendency to establish its breeding season from September- October and November in relation to abattoir genitalia specimens.

3-We can arrange a predictive figure concerning the transition breeding period for ewe in relation to the abattoir specimens nature.

4- It is more convenient results obtained for IVF, oocyte in vitro maturation (IVM), good quality oocyte and to establish a researches or study in sheep is the breeding season.

5- It is better and more accurate time to collect good quality oocytes is breeding season.

6-Oocytes maturation under Iraqi condition needs 30 hours for good events.

7- Collection of good quality oocytes for all assist Reproductive technology activates (ART) is better with breeding season. Ewe oocytes maturation conducted with elevated number within season, and for ART activities it is better to start with breeding season.

8- Abattoir specimen is regarding as a good, easier and cheapest source for oocytes collection

9- Good quality oocytes are the pest choice for IVM.

10- Slicing of the ovaries to small pieces is the best methods for oocytes collection

Discussion:

Most mammals living at temperate latitudes (Iraq) 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. This phenomenon related to the function of Melatonin which secreted only during the night, acts as the endocrine transducer of the photoperiodic message **(Hugues Dardente 2012).).** This research was design to study the effect of day-light season on the sheep cyclicity in relation to the abattoir ovarian samples and to create a good method for the researchers who study the sheep oocytes or In vitro fertilization….. etc to choose the correct season to start their work.

The experimental material of this study are the abattoir specimens, fresh abattoir ewe genital specimens regarded as a main source or reservoir for oocytes, this specimens when collected, preserved in 4-8°C containers and moved directly to the Lab. for further processing was regarded as the main, cheapest and bulky quantity source, or even to be one of a good source for IVM, IVF, ICSI and IVP (**Karami Shabankareh et al.,2011**).

(Sofi et al., 2012) approved that the abattoir materials is the cheapest and most abundant source of immature oocytes for various assisted reproductive technology.

Results shown that; season has a direct effect upon these two parameters in regarding to variations of weight and size, samples collected within breeding season have well developed parameters (**Smith 2012).** agreed with this finding in which; the changing photoperiod acts as a bio-regulator 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 **(Foster 1988).).**

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These physiological changes directly affect the ovarian morphology by increasing follicular development then weight and size of the entire organ (ovaries) will increase, these results agreed with (**Smith and Clarke 2010)**. Seasonal breeding as a neuroendocrine model for puberty in sheep, 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 **(Barrell et al .,1992)**.

Results of this study found that; there is an increasing ovarian weight and size as season changed from short-day light (November) toward long-day light (March), 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), which agreed with **(Karsch,1993).** 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 **(Wood and Loudon 2014).**

The increasing in follicular number and size which start mainly with the ovarian samples, these follicular changes may be due to the changing in the hormonal status of the donor ewes, this is also observed by(**Bartlewski ,1998**), 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 **(Pawel et al .,1999)**.mainly those related to the ovarian samples which were bearing Corpora lutea collected breeding season, and 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.

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 March then it goes down gradually to be equal at August-September (breeding season). This is agreed with **(Peter ,2015)** , 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).

(**Atkinson et al .,1998**) 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.

Lasienè, *et al.,* (**Lasienè et al .,2009**) put her agreement in the maturation of Oocytes, in which, it includes two interrelated processes, maturation of nucleus and cytoplasm, and mammalian oocytes are seen within ovarian follicles at diplotene stage of first meiotic prophase.(**Fan and Sun 2004).**)accept what take place within the event of maturation, and this is mainly due to the

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effect of anterior pituitary LH that oocyte rehabilitates meiosis, nucleus and its membrane arrangement.

(Sun et al .,2009) Agree that the first meiotic division established when first polar body project and enters perivitelline space, 2nd meiotic division star and arrested in metaphase Π, maturation of the oocyte is confirmed

(**Fakhrildin et al .,2013)** Regarded oocyte maturity in depending on the presence or absence of 1st polar body and \or compacted or expanded of COCs is the method for oocyte evaluation.

(**Evans,2003).** approved that the physiology and maturity of donor ewe affect the oocyte development, maturity and the produced embryo quality, and this is (may be) one of many reason that affect the oocytes quality (elevated number of poor and bad oocytes) collected in the study which in turn reflected the oocyte maturation index occurred at breeding season.

The intra- ovarian physiology and activity in regarding to the donor ewe maturity could be affected the oocyte maturity,(**Webb,2007)** approved that kind of internal factor that affect mostly oocyte maturity, in which; slaughtered house ewe genitalia samples have no previous history concerning the donor ewe physiology.

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