Low oocyte quality related with the aging ewes

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Summary

This study was conducted to know the effect of ewe age on oocyte quality as well as the relations between oocyte viability and normal uterine condition. Eighty three (83) reproductive systems of non-pregnant ewes were collected from Al-shulla abattoir. The Total oocytes were aspirated from right ovaries reached 61.45% and 38.55% from left ovaries. Immediately after aspiration, the oocytes were examined by light microscopic and conceded as mature if surrounded completely with cumulus oopherus. While the stained oocytes by trypan blue were conceded as dead oocytes and excluded. According to ewes age the oocytes were classified into (3) groups, the first group ranged between 1-2 years, second group 3-6 years and the third group over 6 years. The total oocyte collection from these groups was 20, 23, 40 oocyte. The results indicated that 14 oocytes (70%), 17(73.91%) and 10(25%) from groups 1, 2, and 3 with cumulus cells, respectively. While the total live oocyte reached to 60. Normal endometrium was observed in 90%, 95% and 80%for 1,2and 3 groups respectively. It was concluded from this study that aged ewes showed low quality oocyte with infertile endometrium.

Keywords: Related age, Ewe, Low oocyte quality.

Introduction

The relationship between maternal age and the increased incidence of oocyte aneuploidies has been studied in several epidemiological studies (1 - 3).

Maternal age influence offspring quality in many species, the maternal aging may promote the development of conditions in adulthood by impacting the early life conditions of the offspring. Deoxy ribonucleic acid damage in germ cells, chromosomal changes, and pregnancy complications which increase with oocyte age and maternal age (4 - 6) and cancer (7). Delayed motherhood is characterized by increased probability of obstetrical complications and prenatal problems (8). Early embryonic mortality may result from poor egg quality in aged female, as shown in the rabbit by the relatively unsuccessful development of blastocysts transferred from older donor to younger foster mothers (9).

Oocytes play a central role in the establishment of embryonic fate. The oocyte quality plays a major role in fertilization process and embryo development (10), therefore the quality of oocytes play an important role in a proper embryo development (11 and 12 .) Cumulus cells are involved in oocytes growth and maturation. Weather cumulus cells interact with the oocyte or with spermatozoa to promote fertilization. There are different possibilities included, the cause of mechanical entrapment of spermatozoa and guide hyper activated spermatozoa towards the oocyte, while preventing abnormal spermatozoa to enter the cumulus matrix. In addition, the cumulus cells create a micro-environment for the spermatozoa which favours their capacitation and penetration into the oocyte, and prevent changes in the oocyte (13). During aging may be induced by dysfunctions of proteosomes and the endoplasmic reticulum (14). Therefore the objective of this study was designed to investigate the oocyte viability in relation to different maternal age and to the histological picture of the endometrial.

Materials and Methods

Eighty three ewes genitalia collected from Alshuala abattoir in Baghdad Province, transmitted to Obstetrics lab at the College of Veterinary Medicine, Baghdad University. Oocytes recovered from follicles by aspiration and stained with trypan blue. Then, all samples were subjected to microscopical examination for determining the dead and alive oocytes. All oocyte without cumulus cells considered as dead oocytes. Uterine biopsy samples were

taken from non pregnant ewes and fixed in a plastic container that contained 10% neutral buffered formalin for stopping post mortem autolysis. Sectioning via microtome with and stained with (hematoxiline–eosin). The age of ewes determined by the teeth according to (15).

Results and Discussion

The oocytes which were aspirated from right ovary reached 51(61. 45 %). While the oocytes which were aspirated from the left reached 32 (38.55%) (Table,1).

Table, 1: The oocytes aspirated from right and left ewe ovaries of different age

Groups	years	Right Ovary	Left Ovary	Total
1	1-2	8	12	20
2	3-6	16	7	23
3	Over6year	27	13	40
Total		51	32	83
Percent		61.45%	38.55%	

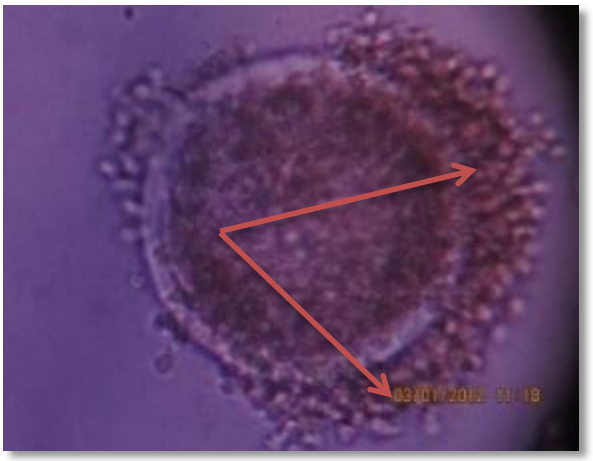
Alwan *et al.*, (16) mention of that the oocytes aspirated from the right ovaries reached 125 while the left ovaries gives 81 in ewes , while the left ovaries reached eighty one in the ewes. In other studies approximately 62% of single ovulation and 56% of double or triple ovulation occur from the right ovary (17 and 18). This indicated that the right ovary of the ewe is more active than left ovaries .

The total harvested oocytes was 83, were classified into three groups according to the age of ewes .The first group ranged between 1-2 years, were 20 oocytes recovered. The second group ranged 3-6 years, were 23 oocytes recovered, and the third group over 6 years was 40 oocytes recovered. Dickerson *et al.*, (19), was classified the ewes in the three groups one year, 4-6 years and 7-9 years.

Dead oocytes in (Fig. 1, 2 and 3) those recovered from aged ewes, showed the fragmentation of cytoplasim and stained by trypan blue. While the oocytes from young showed a uniform cytoplasm, not stained, and without fragmentation**,** in (Fig. 4 and 5).

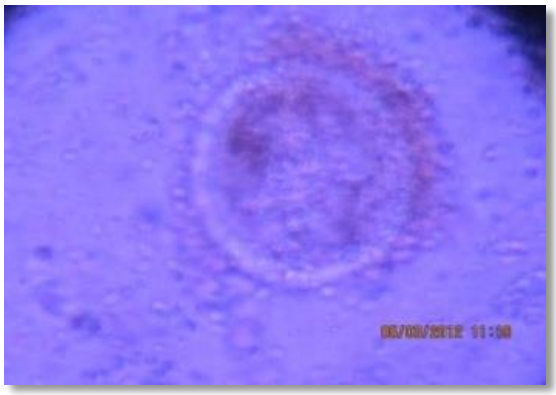
The activities of some material in aged oocytes were lower than those in younger one.

Figure, 1: **oocytes and stained by trypan blue (dead), cytoplasm fragmentation .**

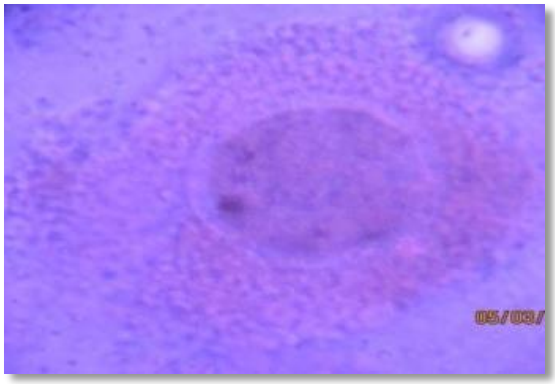


Figure, 2: The different types of cumulus cells arranged around oocytes and stained by trypan blue (dead), cytoplasm fragmentation .

Figure, 3 : **Oocytes without cumulus cells.**



Figure, 4: The (alive) oocytes, uniform cytoplasm without staining by trypan blue, oocyte partial surrounded cumulus cells.



Figure, 5: The (alive) oocytes, uniform cytoplasm without staining by trypan blue, oocyte complete surrounded cumulus cells.

The results was shown in (Table 2,3 and 4) display 85% alive oocytes at 1-2 year, 82,6 % at 3-6 year and 60% at over 6 year old ewe . Dickerson *et al.*, (19), reported the lambing rate measured from 45-75% at one, year 85-95% at 4-6year and reached 60-80% at 9-year-old ewes.

Table, 2: Number of the oocyte (live and dead) related to normal endometrium in ewes aged (1-2) years.

Genitalia Number	Live Oocyte	With Cumulus Cell	Partial Cumulus Cell	Without Cumulus Cell	Normal Endometrium
20	17	14	3	3	18
%	%85	%70	%15	%15	90%

The number of collected oocyte with cumulus cells 14 (70%) in the first group 3 (15%) with partial cumulus cells, and 3 (15%) without cumulus cells. The second group recorded 19 (%82.608) alive oocyte, 17 (%73.913) of this group with complete cumulus cell, 2 (8.695%) with partial cumulus cell , and 4 (17.391%) without cumulus cell (Table, 3).

Table, 3: Number of the oocyte (live and dead) related to normal endometrium in ewes aged (3-6) years.

Genitalia Number	Live Oocyte	With Cumulus Cell	Partial Cumulus Cell	Without Cumulus Cell	Normal Endometrium
23	19	17	2	4	22
%	82.608 %	%73.913	8.695 %	%17.391	95.652 %

Table-4: Number of the oocyte (live and dead) related to normal endometrium in ewes aged (over 6 years)

Genitalia Number	Live Oocyte	With Cumulus Cell	Partial Cumulus Cell	Without Cumulus Cell	Normal Endometriam
40	24	10	13	17	32
%	%60	%25	%32.5	%42.5	80

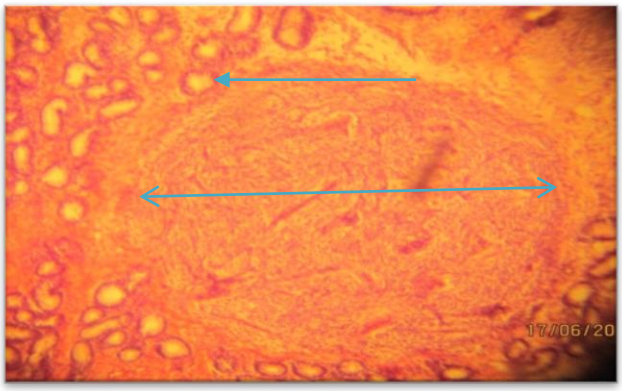
Oocytes of the third group were 24 (60%), 10 (25%) of the alive with complete cumulus cell, 13 (32.5%) with partial cumulus cell and 17 (42.5%) without cumulus cell (Table, 4). The cumulus cells surrounded the oocyte essential for their normal differentiation, regulation, and functions (20). This might be due aging-induced changes in hormonal levels or other physiological parameters that modify the intrauterine environment may influence offspring health (7 and 21).

Genital tract abnormalies included hydrosalpinx with subacute endometritis and chronic endometritis with abscess in the endometrium. The abnormalies in the first group 18, (90%) the second group 22, (95.652%) and the third group 32, (80%).

Histology of endometrium, explain the variation between the three groups. First group included the normal architecture of endometrium, while the second group only two cases appeared subacute endometritis, necrotic endometritis were in agreement with Hatpoglu *et al.*, (22), and hydrosalpinx in the other case. The third group display narrowing in the endometrium, decrease in the number and the size of uterine glands and some vaculation of epithelium with scattered infiltration and diffuse of fibrous connective tissue. The

senility in women the endometrium atrophies, becoming reduced to thin layer while its glands tend to form small cysts.

Uterine aging in part responsible for adecline in fecundity. These include age related changes in the hypothalamus - pitutary, and ovaries loss of number or function of steroid hormone receptors ,morphological changes in the uterine epithelium, the accumulation of collagen fibrils in the uterine stroma, and loss or impairment of the decidual response (23). Sub-acute endometritis display 5% cases infiltrated by plasma cells and some lymphocytes with scattered narrowing uterine gland particularly in the stratum spongeoza (24). Another case is chronic appeared the variant fibrosis distributed around uterine glands, lost the architecture and replaced by necrotic tissue, congested blood vessel with filled by RBCs, Fig. 6.



Figure, 6: Chronic endometritis shows the damage of uterine glands → and occupied by necrotic tissueand surround by fibrous connective tissue ↔ (H and E X 20).

There was no significant differences between dead and live oocytes in different the groups (P>0.05). While there was a significant difference surrounded cells (P<0.01) with different groups.

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تقدم العمر للاغنام وعلاقته بالانخفاض النوعي للبيوض

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الخلاصة

صممت هذه التجربه لدراسة تاثير عمر النعاج على نوعية البيوض و كذلك العلاقة ما بين الرحم وحيوية البيوض .جمعت 83 عينة من الجهاز التناسلي الانثوي من مجزرة الشعلة . كانت نسبة البيوض المجمعة من المبايض اليمنى 61.45 % , اليسرى 38.55% قسمت البيوض اعتمادا على عمر النعاج الى ثلاثة مجايع عمرية , تراوحت اعمار المجموعة الاولى 2-1 سنة , والمجموعة الثانية 6-3 سنوات و المجموعة الثالثة اكثر من 6 سنوات. اما عدد البيوض فكان 20 , 23 و40وعلى التوالي بويضة بتسلسل المجاميع . عند الفحص بلغ عدد البيوض المحاطة كاملا بخلايا الركمة المبيضية 14 في المجموعة الاولى و17 في المجموعة الثانية و10 بيوض في المجموعة الثالثة. بلغ عدد البيوض الحية الكلي (60) اما نسبة الارحام الطبيعية فكانت في المجموعة الاولى 90% و95% في المجموعة الثانية و80% في المجموعة الثالثة . وقد استنتج من الدراسه ان افضل عمر لجمع البيوض من النعاج مابين 6-3 سنوات كما بين الفحص المجهري لطبقات الرحم ان الاصابات الرحميه تزداد بعد السنه السادسه من عمر النعجه فضلا عن عدم وجود فرق احصائي بمستوى (P>0.05) في عدد الخلايا الحية والميتة بينما كان هناك فرق احصائي (P<0.01) في البيوض المحاطة بشكل كامل بالخلايا الركمة في المجموعة 1و2 مقارنه المجموعه 3. نستنتج من هذه الدراسة ان الاغنام المسنة تكون بيوضها اقل نوعية وبطانة رحم قليلة الخصوبة.

الكلمات المفتاحية : تقدم العمر, الانخفاض النوعي للبيوض, الاغنام.