Effect of *Artemisia absinthium* L. and vitamin E supplementation on maturation and in vitro fertilization of local Iraqi sheep oocytes in different seasons

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Abstract

The study was conducted from December 31, 2023, to December 31, 2024. Its aim was to determine the effect of adding Artemisia absinthium L. and Vit. E supplementation on maturation and in vitro fertilization in different seasons. 382 ovaries were collected from ewes slaughterhouses in Fallujah District, Anbar Governorate, yielding 472 ova. The obtained oocytes were divided into four groups: G1 control (DMEM culture medium without additives), G2 (DMEM culture medium + Artemisia absinthium L. 25 µmol/100 ml), G3 (DMEM culture medium + Artemisia absinthium L. 50 µmol/100 ml), and G4 (DMEM culture medium + Vit. E 100 µmol/100 ml). The study results showed significant differences ($P \leq 0.05$) in the distribution of ovarian follicles, with smaller follicles (58.1%) outnumbering larger follicles (41.8%). The right ovary also recorded a greater number of follicles and retrieved eggs compared to the left ovary. Furthermore, the results demonstrated a significant effect of seasonal factors on the number of retrieved oocytes, with autumn recording the highest average number, followed by spring, then winter and summer. G3 resulted in the highest rate of oocytes maturation, followed by G4, then G2, compared to the control G1. Autumn also recorded the highest maturation rate, followed by spring. G3 recorded the highest fertilization rate, followed by G4, then G2, while G1 had the lowest. Autumn and spring also had the highest fertilization rates. The study concludes that adding Artemisia absinthium L. (50 µmol/100 ml) and Vit. E 100 µmol/100 ml effectively contributes to improving the maturation and fertilization efficiency of cultured ova and the early embryonic development of laboratory-produced embryos by reducing oxidative stress. The results also confirm the importance of seasonal factors in improving reproductive efficiency, particularly during the autumn and spring seasons.

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Keywords: Artemisia absinthium L. and Vit. E, maturation, in vitro fertilization, oocytes, Iraqi sheep

Introduction

In vitro fertilization (IVF) is a modern, advanced technique for improving genetic traits and productivity in animal production, including sheep. Its applications have increased significantly in recent years due to the growing demand for enhancing reproductive efficiency and preserving desirable local breeds. Annual data from the International Society for Embryo Transfer (IETS) indicates that in vitro embryo production in small ruminants, including sheep, has increased by more than 60% compared to previous years. This demonstrates technological advancements and the significant global reliance on this technique in breeding and improvement programs [51]. Therefore, this technique is considered a type of reproductive biotechnology that has a high potential for accelerating the improvement of genetic traits in ruminants [52], protecting endangered local breeds, creating disease-free herds, and developing biological research models. This is due to the physiological similarities sheep share with humans, making them an important model in biological and reproductive research studies on humans [13, 45]. These in vitro embryo culture techniques have paved the way for studying mammals under controlled conditions, and this technique has become increasingly common since 1978[17].

In vitro embryo production (IVEP) techniques have emerged using eggs obtained after slaughter at abattoirs or through the process of withdrawing eggs from live females, with the possibility of applying them on a wider scale and transferring them from the laboratory to the field. The use of immature lambs also provides an opportunity to greatly reduce the time gap between one generation and another, which promotes genetic improvement at a faster pace[26, 48].

Oocyte quality is one of the most important determinants of the success of IVEP, as it is affected by complex vital functions including metabolic balance, membrane integrity, and genetic efficiency. Oocytes and embryos are very sensitive cells to environmental conditions, especially oxidative stress, which is one of the most prominent challenges that negatively affect oocyte quality and embryo development. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the ability of cells to suppress or reduce their effects. Free radicals contribute to lipid oxidation, DNA damage, and the stimulation of programmed cell death, which leads to a deterioration in oocyte quality and a decrease in the maturation and fertilization rates of oocytes [2, 42, 46]. This leads to an environment unsuitable for maturation, fertilization, and embryo growth. Hence the need to modify culture media by adding natural

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or artificial antioxidants to compensate for the deficiency and improve the efficiency of maturation, fertilization, and early embryonic division[15].

Numerous studies have demonstrated the effectiveness of natural plant extracts as antioxidants, including Artemisia absinthium L. and Vit E. These natural antioxidants are important for improving the quality of culture media, reducing free radicals, and promoting embryonic development[6]. Other studies have indicated that laboratory environmental conditions during the early stages of embryonic development can induce or cause changes in gene expression, which are reflected in the phenotypic characteristics of offspring [11, 22]. This underscores the importance of optimizing the laboratory culture media environment. Since the effect of Artemisia absinthium L. and Vit E. has not been experimented in sheep, the present study attempted to determine the role of Artemisia absinthium L. and Vit E. in in vitro sheep oocyte maturation and in vitro fertilization in different seasons.

Materials and Methods

Experimental Sample: This study relied on samples taken from the slaughterhouse in Fallujah District, Anbar Governorate, as a source for sheep ovaries for the experiment. 191 female reproductive organs, comprising (382) ovaries, were collected, yielding (472) ova, between December 31, 2023, and December 31, 2024, across the winter, spring, summer, and autumn seasons. The research was conducted in the Reproductive Technology Laboratory, Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Fallujah.

Method of Collection.

Ovaries Collection From Slaughterhouse: The study was conducted on 382 ovaries from local sheep collected from the Fallujah slaughterhouse between December 31, 2023, and December 31, 2024. Some ovaries were discarded due to hypoplasia, while others were found to have polycystic ovaries. The ovaries were transported in a refrigerated container with physiological saline to the Laboratory, within one hour. The ovaries were cleaned and sterilized with physiological saline to remove blood clots and debris. Any adhesions and surrounding connective tissue were then removed with sterile scissors and placed in a sterile glass container. The diameters of the follicles were measured using automatic vernier.

Egg collection from the ovaries: Oocytes were collected using the aspiration method in a UV-sterilized chamber. The ovaries were grasped with sterile forceps and placed in a Petri dish containing normal saline. A G-18 needle was inserted into a 5 ml syringe containing 3 ml of DMEM culture medium through the ovarian surface opposite the follicle. The follicle contents were then aspirated into the syringe containing the culture medium. A small amount of culture medium was then injected into the follicle, and its contents were aspirated a second time to ensure complete removal. After aspiration, the contents of the syringe were transferred to a sterile 16-well

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multi-hole dish containing the culture medium, which was housed in an ethyl alcohol-sterilized chamber along with all instruments. The oocytes were then transferred to an inverted microscope for examination, assessment, and classification to determine the different types before incubation and the addition of antioxidant solutions.

The groups were as follows: Control group (DMEM with no additives; G1), DMEM + 25 µmol/ 100 ml Artemisia absinthium L. (G2), DMEM + 50 µmol/ 100 ml Artemisia absinthium L. (G3) and DMEM + 100 µmol/ 100 ml Vit E. (G4).

Additives used and their preparation: 100 µmol/ 100 ml of Vit. E from the Bulk Supplement.com, USA, was added according to [7]. As for the Artemisia absinthium L. extract, the plant was collected in October 2023. The leaves were picked and dried in a dry place until they were completely dry. The leaves were then ground into a fine powder and stored in paper bags in a dry, moisture-free place. An aqueous extract of Artemisia absinthium L. was prepared by fermenting 200 g of the powdered Artemisia absinthium L. leaves with 1 liter of distilled water for 24 hours at 25°C. The mixture was thoroughly stirred with the water using a hotplate stirrer at 30°C. The mixture was then filtered through filter paper into a sterile glass container until the filtrate was completely dry. The container was then tightly sealed. The filtrate extract was then placed in an air purifier to filter out any remaining particles and to sterilize it using a Nalgene filter (0.45 µm) to ensure it was suitable for use. It was then stored in test tubes in a refrigerator at 5°C, according to the method described by [34].

Preparation of the Minimum Essential Medium (MEM): The Minimum Essential Medium (MEM), produced by Gibco, USA, was utilized in this study. Its preparation followed the guidelines provided by the manufacturer.

Oocytes evaluation: The oocytes collected from the slaughterhouse by aspiration were examined under an inverted microscope and classified as good (A), fair (B), and poor (C) based on the number and layers of cumulus cells and cytoplasmic homogeneity, according to [49].

In vitro maturation of Oocytes: After collection, the oocytes were washed in MEM culture medium and then incubated in a Well × 16 dish containing the culture medium. Two groups were added: Artemisia absinthium L. extract (25 µmol/100 ml) and Artemisia absinthium L. extract (50 µmol/100 ml), and Vit. E (100 µmol/100 ml). The fourth groubs was left untouched. The dish was then incubated at 38.5°C, 5% CO2, and 90% relative humidity for 24 hours. After maturation, the dishes were examined under an inverted microscope. The first indicator of successful in vitro maturation (IVM) of oocytes is the appearance of the first polar body. The number of mature oocytes was then counted.

Semen collection: Semen was collected from a ram with high reproductive

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characteristics using electroejaculator manufactured by Anicam Enterprises Inc. The semen samples were collected in 15 ml graduated tubes and immediately transported to the laboratory. They were then placed in a water bath at 37°C and diluted 1:40 with MEM culture medium. Ten micromoles of heparin were then added to the diluted semen to ensure sperm adaptation for fertilization. The diluted semen was then incubated at 35°C for half an hour to complete sperm adaptation.

in vitro fertilization: 24 hours after the maturation process is complete, 50% of the culture medium is withdrawn from the dish containing the mature oocytes using a micropipe, and 50% of the culture medium containing the required antioxidants is added. After microscopic examination confirming oocyte maturation and sperm adaptation, sperm at a concentration of 1×10^4 cells/ml is added to each well containing 3 ml of culture medium. The multi-plate is then incubated at 38.5°C, 5% CO_2 , and 90% relative humidity for 30 hours. The fertilized oocytes are then cultured in the culture media used in this study, examined under a microscope, and returned to the incubator. Embryonic development is monitored every 24 hours, and oocytes that do not exhibit the next division are removed from the dishes containing the developing embryos. This process is repeated with each daily media exchange. The percentages of fertilized oocytes that reached the 2-cell to 4-cell stage were observed within 48 hours, morula at 120 hours, blastocyst at 168 hours, and blastocyst expansion at 216 hours after fertilization.

Statistical analysis: The Statistical Analysis System (SAS 2012) [39] was used to analyze the data to study the effect of different factors on the studied traits according to a factorial experiment (4 × 2) that applied a completely randomized design (the mathematical model below). Significant differences between means were compared with Duncan's (1955) polynomial test[9], and the Chi-Square test (χ^2) was used to compare significant differences between percentages.

Result and Discussion

Effect of ovarian type on the number and diameter of follicles:

Table 1 shows that small follicles significantly outnumbered large follicles ($P \leq 0.05$) in both number and diameter. Approximately 466 small follicles were extracted, representing 58.1% of the total, with an average diameter of 3.27 ± 0.03 mm. In contrast, 336 large follicles were extracted, representing 41.8%, with an average diameter of 5.90 ± 0.09 mm. This higher number of small follicles is attributed to the fact that one or more follicles often develop into dominant follicles that secrete estrogen [14]. The results are consistent with [1], whose findings showed a significant advantage in the number and diameter of small follicles over large ones. Specifically, 59.14% of the follicles obtained were small (2-4 mm) with an average diameter of 3.28 ± 0.03 mm, while 40.86% were large (5-8 mm) with an average diameter of 5.44 ± 0.08 mm. These results also align with [29], who

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found that small follicles (2-4 mm) were more successful than larger ones (5-8 mm).

Numerous studies have demonstrated a correlation between follicle size and oocyte quality. Oocyte quality increases with follicle size due to the provision of more favorable conditions. Furthermore, the optimal size for highly viable cultured oocytes is often found between small and large follicles [19] as these follicles are exposed to greater hormonal stimulation and growth factors [50]. Additionally, follicle size plays a significant role in the quality of oocytes retrieved during ovulation[43]. These findings are consistent with[18, 23], who indicated that follicle size can be influenced by several factors, including the animal's reproductive status, breeding season, age, and hormonal stimulation.

Table (1) Number and size of follicles obtained from the ovaries

Follicle size	No.	%	Mean ± SE (mm)
Large	336	41.8	5.90 ± 0.09 A
Small	466	58.1	3.27 ± 0.03 B
Total	802	100%	-
χ^2 **(P-value)**	21.08* (0.0001)		-

Different large letters reveal a significant difference (P<0.05).

The number and percentage distribution of follicles and ova according to the type of ovary:

Table (2) shows the effect of ovarian position on the number of follicles and ova obtained. The number of follicles was counted in 382 ovaries, and the total number of follicles was 802. A numerical increase was observed in the right ovary compared to the left ovary, as the right ovary contained 418 follicles, which represents 52.11% of the total, while the number of follicles in the left ovary was 384 follicles, or 47.88% of the total. However, this difference did not reach the level of statistical significance at the (P>0.05) level. Several recent studies have indicated that the number of follicles is not an accurate indicator of reproductive efficiency due to its dynamic nature and its susceptibility to numerous physiological factors [20]. In contrast, the number of oocytes is a more accurate indicator, reflecting follicular maturation efficiency and the quality of the follicle's internal environment. Oocyte functional efficiency is acquired during its developmental stages within the follicle [10, 53]. The results also showed statistically significant differences ($P \leq 0.05$) in the number of oocytes retrieved between the two ovaries. The right ovary was superior to the left in the number of oocytes retrieved from the follicles, with a total of 269 oocytes (56.99%) from the right ovary,

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compared to 203 oocytes (43.01%) from the left ovary.

The study's results are consistent with [1], who observed a significant difference in the percentage of follicles in the right ovary (58.13% of the total), compared to 41.87% in the left ovary. The results also showed significant differences in the number of eggs retrieved between the right and left ovaries, with the right ovary yielding 776 eggs compared to 592 eggs from the left.

These results are also consistent with [23, 30], whose data showed that the left ovary exhibited less activity compared to the right ovary in terms of the number of follicles. Significant differences were also observed between the two types of ovaries. Furthermore, the study by [41] confirmed that the right ovary was more active than the left, as a higher number of ovulations were observed in the right ovary compared to the left in ewes. Moreover, these results did not agree with [29], who demonstrated that the left ovary (59.4%) was superior to the right (40.6%) by a statistically significant difference ($P \leq 0.05$) in terms of the number of follicles and collected ova. The results also contradicted that the left ovary yielded more eggs than the right, despite no significant difference in the number of follicles retrieved, due to the egg retrieval method used. The difference in perceived activity between the right and left ovaries may be attributed to several factors, including lineage and ovarian blood supply, as noted by [41]. Furthermore, the number and quality of retrieved eggs vary depending on the retrieval method. Sogorescu et al. [44] indicated that ova retrieved via aspiration are of higher quality compared to other retrieval methods.

Table (2) Number and percentage of follicles and ova vary depending on the type of ovary.

Ovarian type	Number of samples	Number of follicles	Number of ova
Right	195 (51.05%)	418 (52.11%)	269 (56.99%)
Left	187 (48.95%)	384 (47.88%)	203 (43.01%)
Total	382	801	472
χ^2 (P-value)	0.1675 NS (0.6823)	1.441 NS (0.2299)	9.229** (0.0024)

Different large letters reveal a significant difference (P<0.01), NS: Non significant

Effect of season on the number and diameter of follicles obtained and the average oocyte rate:

Table (3) illustrates the effect of season on follicle diameter, follicle size, and average oocyte count collected during

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the research period. The results showed significant effects of season (P≤0.05) on follicle size, follicle diameter, and average oocyte count, with varying averages depending on the season. This indicates that seasonal environmental factors play a key role in regulating ovarian physiology.

In the diameter of the follicles, the highest average was recorded during autumn, reaching 6.15±0.11 mm, which significantly surpassed the rest of the seasons. This was followed by winter and spring, which recorded 4.52±0.38 mm and 4.34±0.33 mm respectively, without significant differences between them. Meanwhile, summer recorded the lowest average, with a mean of 2.36±0.36 mm. This variation between autumn and the rest of the seasons may be attributed to the temperature and moderate environmental conditions in this season, which leads to an improvement in the efficiency of hormonal secretion, especially an increase in the secretion of LH and FSH hormones that stimulate the ovaries, leading to the promotion of follicle growth. In contrast, heat stress during the summer causes inhibition of ovarian activity and a decrease in the growth rate of follicles as a result of the disturbance that occurs in the hormonal balance and an increase in the secretion of stress hormones such as cortisol [28].

As for the number of follicles, the highest average number of follicles was in autumn, reaching 83.67±2.73, followed by winter with an average of 68.00±10.02, then spring with 63.00±7.21, and there were no significant differences between them. Meanwhile, summer recorded the lowest average number of follicles, reaching 52.67±9.52, which confirms the same seasonal effect of follicle diameter, as the increase in the number of follicles is associated with improved environmental conditions and ovulation efficiency during moderate conditions, while the number decreases due to the negative effects caused by high heat stress, which in turn reduces the efficiency of growth and development of ovarian follicles [54].

The results of this study are consistent with many previous studies and research that mentioned the existence of clear seasonal effects on reproductive activity, as it showed that in temperate seasons there is an improvement in the characteristics of ovarian follicles compared to hot seasons. These studies attributed this to improved food consumption, reduced heat stress, and an improved environment for internal hormones. The studies also showed a reduction in the sensitivity of the ovary to stimulating or ovulation-stimulating hormones during high temperatures, which leads to a decrease in the number and diameter of follicles[47].

This is followed by spring with a relatively good average of 10.66±1.10, then winter with a relatively lower average of 10.00±0.24, compared to summer, which recorded a decrease in the average rate of mature ova, reaching 6.00±0.94. This can be explained by the severe environmental stresses that animals may be exposed to during this season, which may negatively affect the quality of ova obtained from the ovaries [36]. Oxidative stress (ROS) occurs

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when the production of reactive oxygen species and other radical species exceeds the ability of antioxidants to eliminate them, due to the overproduction of reactive oxygen species and the disruption of antioxidant enzymes[6].

These results also indicate a clear effect of seasonal factors on reproductive activity, and this may be explained by temperature and heat stress, which may negatively affect the growth of ovarian follicles. Heat stress leads to changes in the follicular environment within the ovary, causing a disturbance in follicular growth. A recent study indicated that heat stress leads to changes in follicular fluid and affects the efficiency of obtained eggs and the development of embryos in the laboratory as a result of disturbances in metabolic and hormonal indicators within the follicle[21].

On the other hand, elevated temperatures affect the granulosa cells surrounding the oocyte within the ovarian follicle, leading to a decrease in the secretion of steroid hormones crucial for follicle growth, such as estrogen and progesterone. This also results in increased apoptosis (programmed cell death) and elevated free radical levels, negatively impacting oocyte maturation and quality [37]. Furthermore, a recent study demonstrated that seasonal variations negatively affect gene expression in oocytes and granulosa cells due to environmental changes, particularly temperature fluctuations. A decrease in the percentage of high-quality oocytes was observed during the hot season compared to the temperate season[16].

These results are consistent with [32, 54] who stated that summer is the most influential season on animal fertility due to heat stress, which causes changes in follicle development and reproductive hormone secretion, leading to a decrease in follicle number and efficiency. This clearly indicates a decline in fertility or cessation of reproductive activity during the summer and winter seasons. This may be due to fluctuations in temperature and the accompanying physiological stresses that affect ovulation performance or the quality of extracted samples.

The results of this study are consistent with several other studies that have indicated a clear seasonal effect on fertility and reproductive activity in animals. Serag El Din et al. [40] reported that reproductive activity is highest during the autumn and spring seasons due to moderate environmental conditions, while it is lower during the summer and winter due to heat stress or extreme cold and their effect on reproductive hormone secretion.

As Batool et al. [4] explained, high temperature and humidity levels lead to decreased LH and FSH secretion and delayed ovulation, resulting in lower fertility rates during the summer and winter seasons. Furthermore, heat stress affects ovarian follicle dynamics, leading to disruptions in the growth of the dominant follicle during hot periods. Several previous studies have also shown that sheep breeding seasons coincide with specific times of the year, resulting in lambing in the autumn and

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spring, which provides optimal conditions for lamb growth. Regulating sheep reproductive activity at specific times of the year is crucial for successful breeding. This aligns with the findings [24], who noted a significant difference in the number of follicles obtained during the breeding season compared to those collected outside of it. According to Bronson [5], this seasonal pattern of reproduction is linked to a range of environmental factors, most importantly the availability of nutrients necessary for follicle growth, the duration of daylight, and geographical location. These variations may be due to the influence of seasonal temperature changes. Moderate temperatures during the autumn season contribute positively to cell maturation, while significant temperature fluctuations, whether increases or decreases, can negatively affect follicle growth and development [31]. Autumn is considered the ideal season for ovarian activity in terms of the growth and number of follicles and mature ova obtained. In contrast, summer is less efficient in the number and diameter of follicles obtained [35]. This may be explained by the fact that in the moderate seasons in the climate of some countries, such as Iraq, the reproductive activity of sheep begins, and therefore the ovaries are active and there are growing and developing follicles, with the availability of food that affects the readiness of females to reproduce[25].

Table (3) Effect of the season in diameter, number and rate the follicles of ova retrieved

Season	Follicles diameter (mm)	Number of follicles	Rate of Oocyte
Winter	4.52 ± 0.38 B	68 ± 10.02 AB	10 ± 0.24 A
Spring	4.34 ± 0.33 B	63 ± 7.21 AB	10.66 ± 1.10 A
Summer	2.36 ± 0.36 C	52.67 ± 9.52 B	6 ± 0.94 B
Autumn	6.15 ± 0.11 A	83.67 ± 2.73 A	12.66 ± 0.48 A

Different large letters reveal a significant difference ($P<0.05$).

Effect of additives, season, and their interaction on the maturation rate:

Table (4) showed significant differences in the rate of egg maturation based on the different groups used and the seasons. The control group recorded the lowest maturation rates over the different seasons with an average of 6.75 ± 0.82. In contrast, groups G2, G3 and G4 showed higher maturation rates ranging between 9.41 ± 1.15, 9.08 ± 1.13 and 8.58 ± 1.04, which indicates

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the effectiveness of the additives in promoting the growth maturation environment in the culture medium and activating the vital processes associated with egg maturation (Fig. 1). Regarding the effect of seasons on the maturation rate, the results showed that autumn had the best overall oocyte maturation rate, reaching 11.50 ± 0.58, followed by spring and winter, with rates of 9.08 ± 1.15 and 8.16 ± 0.53 respectively, without any significant differences between them. Finally, summer recorded the lowest values, at 5.08 ± 0.94. The lower maturation rate in summer may be attributed to higher temperatures, which can negatively affect protein structure or cell membranes, thus limiting the effectiveness of the culture medium and reducing the chances of complete oocyte maturation. The results also indicate that spring and autumn provide ideal conditions to support oocyte maturation in vitro [25].

The study demonstrated a clear interaction effect between season and application groups. G3 achieved the highest maturation rate during autumn (12.66 ± 1.20), followed by G4 in the same season (11.50 ± 0.58). This improvement is attributed to the positive effect of antioxidants Vit. E and plant compounds (wormwood extract) in protecting cells from oxidative stress and stimulating cell maturation, especially under favorable environmental conditions such as those found in spring [27].

Table (4) Effect of additive and seasonal coefficients and their interactions on the rate of oocyte maturation

Groups	Season				
	Winter	Spring	Summer	Autumn	Mean of Groups
G1	6 ± 0.57 bcd	7.66 ± 0.66 abcd	3.66 ± 1.76 d	9.66 ± 1.2 abcd	6.75 ± 0.82 A
G2	8 ± 0.57 abcd	9.66 ± 2.33 abcd	5 ± 2.08 d	11.66 ± 1.33 abc	8.58 ± 1.04 A
G3	9.66 ± 0.88 abcd	9.66 ± 3.33 abcd	5.66 ± 1.85 cd	12.66 ± 1.20 a	9.41 ± 1.15 A
G4	9 ± 1.0 abcd	9.33 ± 3.28 abcd	6 ± 2.64 bcd	12 ± 0.57 ab	9.08 ± 1.13 A
Mean of Season	8.16 ± 0.53 A	9.08 ± 1.15 AB	5.08 ± 0.94 C	11.50 ± 0.58 A	

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Different large letters reveal a significant difference between groups (P<0.05).
Different small letters indicate overlap between seasons and groups (P<0.05).

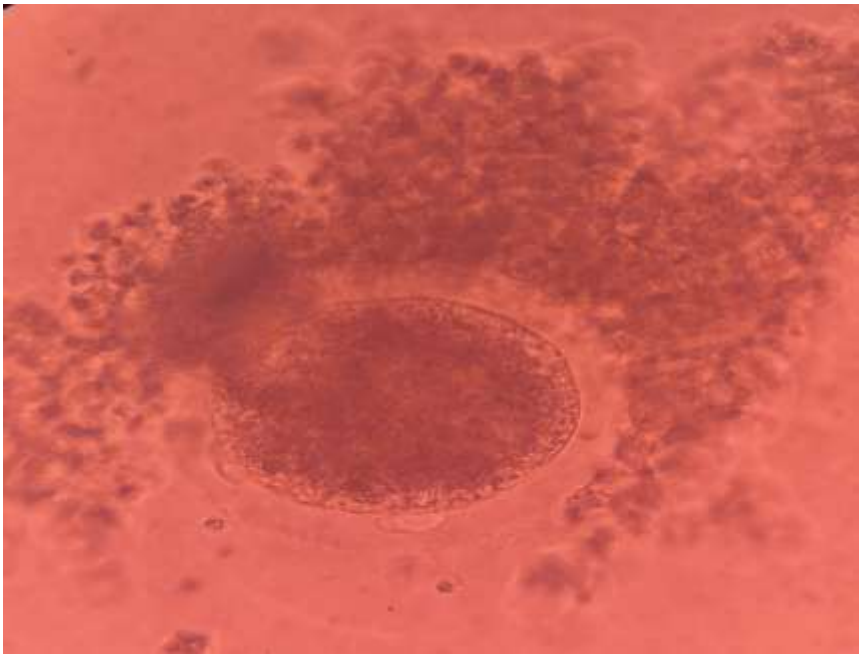

Fig. (1) Mature oocyte

Effect of adding antioxidants on oocyte fertilization rate:

Table (5) shows clear and significant differences in fertilization rates between the different groups, in addition to the effect of season and interaction between factors. The data showed that G1 recorded the lowest fertilization rate compared to the other groups. Conversely, all other groups achieved significant results in terms of the ova reaching the fertilization stage (Fig. 2). G3 recorded the highest rate at 9.16 ± 1.05, followed by G4 with an average of 8.50 ± 1.28, while G2 had an average of 8.08 ± 1.22.

These results reflect the effectiveness of natural plant stimulants and antioxidants in reproductive efficiency. This effect is likely due to the role of these compounds in reducing oxidative stress and enhancing the physiological activity of cells involved in fertilization. This also underscores the essential role of antioxidants and their availability[3]. Furthermore, oxidative damage affects not only the oocytes in culture but also the seminal fluid, significantly impacting fertilization rates and ultimately halting cell division. This is confirmed by the significant differences observed between treatments (Ribas-Maynou and Benet, 2019). Various studies have demonstrated that oxidative stress poses a significant problem due to the high sensitivity of

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sperm cells to elevated levels of reactive oxygen species. This leads to defects in sperm cells, such as DNA damage, protein alterations, and plasma membrane damage [6, 8].

Regarding the effect of season, the results indicate that the fertilization rate reached its highest levels during the autumn season, with an average of (10.08 ± 0.94), followed by spring (8.66 ± 1.37), with a clear and significant difference compared to the winter (7.08 ± 0.82) and summer (4 ± 0.76) seasons. This is attributed to the different climatic conditions of each season, including temperature, humidity, and light intensity, which directly affect physiological activity and the effectiveness of the culture medium. The observed decrease in the fertilization rate during the summer is likely due to the higher temperatures and the associated heat stress, which negatively impacts the cells or the surrounding environment [1].

The interaction between groups and season showed that the highest fertilization rate was recorded in G3 during autumn (12 ± 0.57), followed by G4 in the same season (11.66 ± 1.20), confirming that the effectiveness of additives is more pronounced under suitable climatic conditions. Thus, it is clear that the interaction between groups type and season is a crucial factor in determining the success or effectiveness of groups, necessitating consideration of the season when implementing such experiments or improvement programs. The study observed that the fertilization rate was affected by the reproductive season. These results align with [38], who indicated that treating semen with antioxidants in the fertilization medium improved semen characteristics, including plasma membrane integrity, motility, and fertilization rate. The results are consistent with the findings of researcher [12], who explained that treating rams with antioxidants reduced levels of reactive oxygen species (ROS) and improved the characteristics of semen by protecting it from oxidative stress damage. The results are not consistent with [1] indicated, as he did not observe any statistically significant differences between the study treatments during the fertilization stage of sheep oocyte.

The current study concludes that the addition of Artemisia absinthium L. and vitamin E plays a significant role in oocyte maturation and fertilization. Furthermore, the influence of seasons and the interaction between groups and season are important factors in maturation and fertilization. This underscores the importance of considering environmental conditions when designing or implementing oocyte maturation programs in practical settings.

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Table (5) Effect of additive factors, season, and their interaction on the rate of ovum fertilization

Groups	Season				
	Winter	Spring	Summer	Autumn	Mean of Groups
G1	3.33 ± 0.33 ef	4.66 ± 1.20 edf	2.66 ± 0.88 f	5.66 ± 0.66 bcdef	4.08 ± 0.49 B
G2	7.33 ± 0.66 abcdef	10.33 ± 2.66 abcd	3.66 ± 2.02 ef	11 ± 2.08 abc	8.08 ± 1.22 A
G3	9 ± 0.57 abcde	10.33 ± 2.90 abcd	5.33 ± 1.76 cdef	12 ± 0.57 a	9.16 ± 1.05 A
G4	8.66 ± 2.02 abcdef	9.33 ± 3.66 abcde	4.33 ± 1.76 edf	11.66 ± 1.20 ab	8.50 ± 1.28 A
Mean of Season	7.08 ± 0.82 B	8.66 ± 1.37 AB	4 ± 0.76 C	10.08 ± 0.94 A	

Different large letters reveal a significant difference between groups (P<0.05).
Different small letters indicate overlap between seasons and groups (P<0.05).

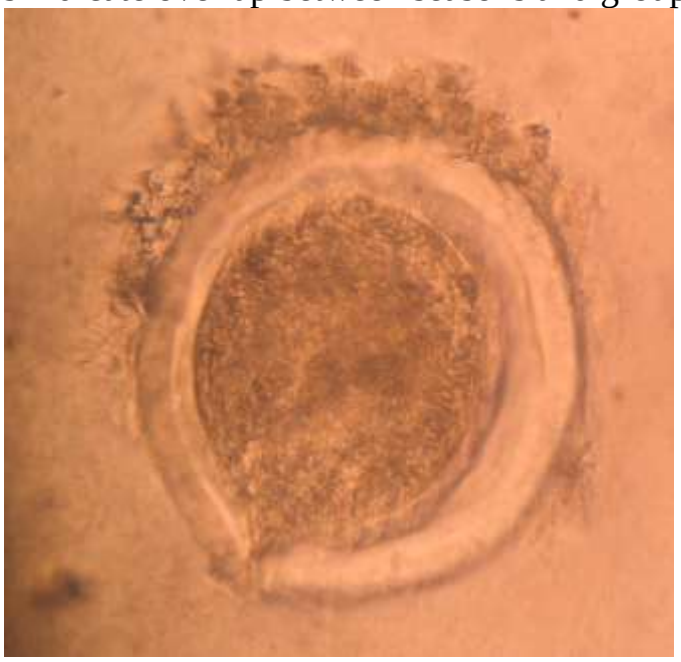

Fig. (2) Egg fertilization stage

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