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Study of Some Biochemical and Vitamins Changes in the Occurrence of Repeated Fertilization Failure in Sheep

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Abstract

The current study aimed to diagnose the most important causes of fertilization failure in ewes and the relationship of some biochemical parameters to their occurrence. The study included 40 sheep, their ages ranged between (1-5) years, from different areas of the outskirts of the city of Tikrit and for the period that lasted from March/2022 to September/2022. The animals were divided into two groups, the first group (30) sheep suffering from fertilization failure (Repeated return to estrus more than three times, and pregnancy was not recorded in these ewes during the current reproductive season), while the second group (10) ewes that were pregnant and healthy were considered as a control group. Blood samples were taken from all study animals and serum was prepared from it to measure the level of some biochemical parameters, elements and vitamins in the blood serum, which included glucose, cholesterol, total protein, zinc, copper, selenium, vitamins A and E.

The study showed that there was no significant difference ($P \leq 0.05$) in the level of glucose, cholesterol and total protein in the affected ewes suffering from fertilization failure and frequent estrus when compared with its level in pregnant healthy ewes. As for the levels of elements in the serum, the study noted a significant decrease ($P \leq 0.05$) in the level of zinc concentration in non-pregnant infected ewes compared with the control group, while the study did not notice any significant differences in the levels of copper and selenium concentrations in infected ewes compared to healthy pregnant ewes. The study also noted a significant ($P \leq 0.05$) decrease in the level of concentrations of some vitamins included in the study, as it showed a significant ($P \leq 0.05$) decrease in the level of vitamin A and E concentrations in infected ewes compared to healthy pregnant ewes. We conclude from the current study that the deficiency in zinc and vitamins A and E plays an important role in the occurrence of fertilization failures and maintaining the integrity of reproductive efficiency in ewes.

Introduction

Ewes are considered one of the most important livestock ruminants that take the first place in the animal population in the world (Esteves *et al*., 2018). reproductive problems experienced by ewes may lead to a temporary loss of their reproductive ability due to temporary or permanent sterility that leads to a decrease in the level of production and reproduction in ewes (Noakes *et al*., 2018). fertilization failure plays a major role in reducing of pregnancy rate in ewes, especially during the breeding season, as there are several reasons that contribute to reducing the chance of fertilization in ewes during the reproductive season, administrative, nutritional and pathological reasons (Ali *et al*., 2019). fertilization failure

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occurs in sheep at a rate of 3 to 10% worldwide (Goumenou *et al*., 2003), and inadequate nutrition in minerals and vitamins A and E leads to disruption of endocrine functions (Bartlett *et al*., 1986). vitamins A It is a fat-soluble vitamin that is known to regulate development, cellular growth, differentiation and tissue function, affecting ovarian follicle growth and uterine environment (Scramuzzal *et al*., 2006). Vitamin E is an important micronutrient in the body of ruminants that plays a very important role in the health of ewes and the development of the fetus (Bastani *et al*., 2011). It is an essential fat-soluble nutrient and functions as a lipid antioxidant and anti-sterility agent essential for reproduction (Takada *et al*., 2010).so the current study aimed to: Studying the effect of some biochemical parameters and vitamins (A, E) on pregnancy failure in ewes.

Material and methods

Animals: The study included (30) ewes of a local breed that were not became pregnant during the reproductive season (March 2022 and August 2022), all ewes were return to estrus more than three estrus sycle, and pregnancy was not recorded in these ewes during the season and 10 ewes were success to pregnancy as control group.

Blood samples:

5 ml of Blood samples were collect from the jugular vein the serum were separated using a centrifuge 2500 rpm for 10 minutes then kept at a temperature of (-20) C until biochemical tests are performed. Glucose, cholesterol, proteins, zinc, copper, selenium were measured using spectrophotometer by using special kit (Colorimetric method) according to (Ali, 2017) and the vitamins A and E were estimated using ELIZA technique with special kit (Biotik /VA) according to (Brigelius-Flohe *et al*., 1999).

Statistical analysis:

The results were statistically analyzed using the ready-made program (SPSS) for the values that represent the mean and standard error, and the data were analyzed using the Anova Analysis of variance one way test, and the differences between the totals were determined using the Duncan polynomial test. Tests at the probability level ($P \leq 0.05$) (Al-Jassar. 2017).

Results and discussion

biochemical parameters: The results of the current study, as shown in Table (1), showed that there was no significant difference ($P \leq 0.05$) in glucose concentration in ewes that suffered from infertility failure compared to healthy animals (102.87 ± 3.59 and 122.44 ± 4.10 mg/dl respectively) and these results agree with what was mentioned by Can *et al*., (2007) in sheep, and did not agree with what was mentioned by researcher (Zeitoun, 2022) in camels, Barson *et al*., (2019) ; Ahmed *et al*., (2017) In cows, where it was shown that the low level of glucose in the blood comes as a result of increased peripheral glucose uptake, failure of gluconeogenesis or glycogenolysis, and hyperinsulinemia leads to problems in the secretion of reproductive hormones, which leads to problems in fertilization and the reason is due to the quality of the animal. Studies have proven that the injection of ewes Glucose during the late luteal phase of the estrous cycle increased ovulation rate, FSH secretion, and

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metabolic hormones (Downing *et al*., 1995). The results of the current study showed that there was no difference (P≤0.05) in the level of cholesterol in ewes that were suffering from fertilization failure and healthy ewes (165.11 ± 6.50 and 162.21 ± 5.57 mg/dL, respectively), and this is consistent with what was found by Ahmed *et al*. , (2017) in cows, and the findings of the researchers (Samanta *et al*., 2005) in cows did not agree, and the reason for this difference may be due to the type of animal and physiological status, such as lactation and the type and quantity of feed Introduction to Animals (Guedon *et al*., 1999). The results also showed that there was no significant difference in the level of total protein for ewes suffering from failure of fertilization compared with healthy ewes (4.59 ± 0.21 and 4.63 ± 0.12 mg/dL, respectively) and these results were in agreement with what was stated (Rashed *et al*., 2019). In cows, while it did not agree with what was stated by researchers, Ahmed *et al*., (2017), where they noticed a significant difference in the percentage of total protein in the blood serum of cows.

Table (1): Concentration of glucose, cholesterol and total protein in infertile and healthy animals.

Standards Totals	glucose (mg/dL)	cholesterol (mg/dL)	total protein (g/dL)
Infected animal	102.87±3.59 [a]	165.11±6.50 [a]	4.59±0.21 [a]
healthy animals	112.44±4.10 [a]	162.21±5.57 [a]	4.63±0.12 [a]

The vertically different letters between the two groups mean that there is a significant difference at the level of probability (P≤0.05) of the values, expressed as standard error ± average.

minerals elements:

The results of the current study, as shown in Table (2), showed a significant decrease (P≤0.05) in the level of zinc in ewes that were suffering from infertility failure (3.41 ± 0.07 mg/dL), compared with its level in healthy ewes (3.69 ± 0.09 mg/dL), and these results are consistent with (Ali, 2022 ; Barui *et al*., 2015 ; Campbell *et al*., 1998) in cattle. Zinc plays a major role in maintaining the integrity of the genital epithelium, and is necessary for the implantation of the fetus into the womb (Robinson *et al*., 2006), and low zinc concentration leads to complications such as impaired secretion of hormones, ovarian atrophy, estrous cycle disorder and recurrent miscarriages. In cows (Kotanidis *et al*., 2013). Sarkar, (2006) notes that fertilization failure can also be due to a deficiency of minerals, especially zinc deficiency, which are necessary for the initiation and maintenance of ovarian activity and the release of mature eggs, as zinc contains an antioxidant (Ebisch *et al*., 2007). The results of the current study, as shown in Table (2), showed that there

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was no significant difference (P≤0.05) in the level of copper in ewes that had failed fertilization (52.3 ± 0.72 mg/dL) compared to its level in healthy ewes (54.48 ± 0.82mg/dL). and this agrees with what was found by (Ahmed *et al*., 2017) in cows, while it did not agree with (Ali, 2022). Copper has a key role in being a cofactor for enzymes such as (amine oxidase, copper-dependent superoxide dismutase, cytochrome oxidase and tyrosinase). Copper also plays an important role in fertility (Ingraham *et al*., 1987).

The results of the current study showed a slight decrease in the level of selenium element, but it was not significant in the blood serum of ewes suffering from infertility failure (86.38 ± 1.33 mg/dl) compared to its level in healthy ewes (86.14 ± 2.89 mg/dl), as shown in Table (2), and these results are in agreement with what was reached (Barui *et al*., 2015).

Table (2): Concentration of zinc, copper and selenium in infertile and healthy animals.

Standards Totals	zinc (µg/dL)	selenium (µg/dL)	copper (µg/dL)
Infected animal	3.41±0.07 [b]	52.3±0.72 [a]	86.38±1.33 [a]
healthy animals	3.69±0.09 [a]	54.48±0.82 [a]	86.14±2.89 [a]

The vertically different letters between the two groups mean that there is a significant difference at the level of probability (P≤0.05). The values are expressed as standard error ± average.

Vitamin A:

The results of the current study, as shown in Figure (1), showed a significant decrease (P≥0.05) in the average concentration of vitamin A in ewes that were suffering from fertilization failure (22.82 ± 0.68µg/ml) compared to its level in healthy ewes.(5.22 ± 0.28µg/ml), and these results are in agreement with the findings of (Ceylan *et al*., 2007) in cows, where vitamin A deficiency affects the growth of ovarian follicles, and the environment of the uterus, as it is necessary to maintain the integrity of tissues in the reproductive system (Scramuzzal *et al*., 2006). Where Haliloglu *et al*., (2002) indicated that there is a clear relationship between the concentration of estrogen in the blood plasma with the level of vitamin A during the follicular phase in the estrus cycle in ewes, as Arikan and Rodway, (2000) indicated that vitamin A plays an important role in the synthesis of The hormone progesterone from the luteal cells of the corpus luteum. This is consistent with the findings of Gore, (2016). Which indicated that the level of progesterone hormone increased during the luteal phase period in female goats that were fed beta-carotene.

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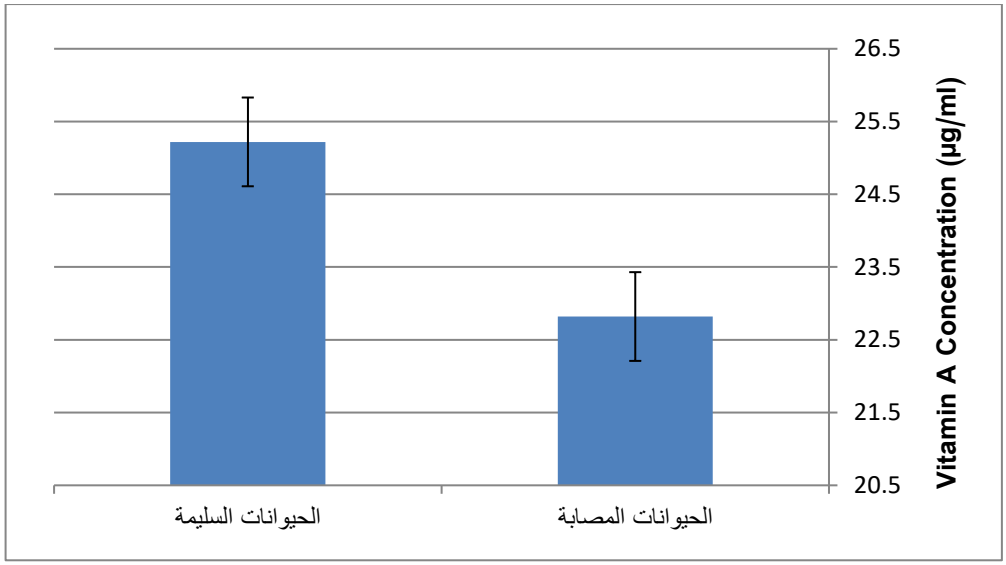

Figure (1): Vitamin A concentration in ewes that suffer from infertility failure and that are healthy. The values are expressed as standard error ± average.

Vitamin E:

The results of the current study, as shown in Figure (2), showed a significant decrease (P≥0.05) in the level of vitamin E concentration in ewes that were suffering from fertilization failure (2.85 ± 0.15µg/ml) compared to its level in healthy ewes (3.44 ± 0.06µg), the result agreed with what was found (Campbell *et al*., 1998) in cows. Where it was observed that vitamin E deficiency has a negative effect on ovulation rates, the results of the current study did not agree with (Musa *et al*., 2018), where it was mentioned that there was no significant change in the level of vitamin E on the fertility rate in sheep. Vitamin E has an important effect on the fertility of ewes, as it acts as an intracellular antioxidant and maintains the integrity of cell membrane phospholipids against oxidative changes, regulating reactive oxygen and thus protecting cell membranes from oxidative damage (Segerson *et al*., 2008), as the reactive oxygen compounds participate in the formation of ovarian follicles, the maturation of eggs, the formation of the corpus luteum, implantation and development of the fetus, oxidative stress has been associated with fertilization failure, implantation failure and early embryonic death (Cetin *et al*., 2010), where the reactive oxygen compounds are: (Reactive oxygen species) are by-products of metabolic metabolism, and play important roles in cellular signaling and cell homeostasis, which suggests that reactive oxygen compounds may have a dual role in fertilization (adverse or protective factors) (Edreva, 2005).

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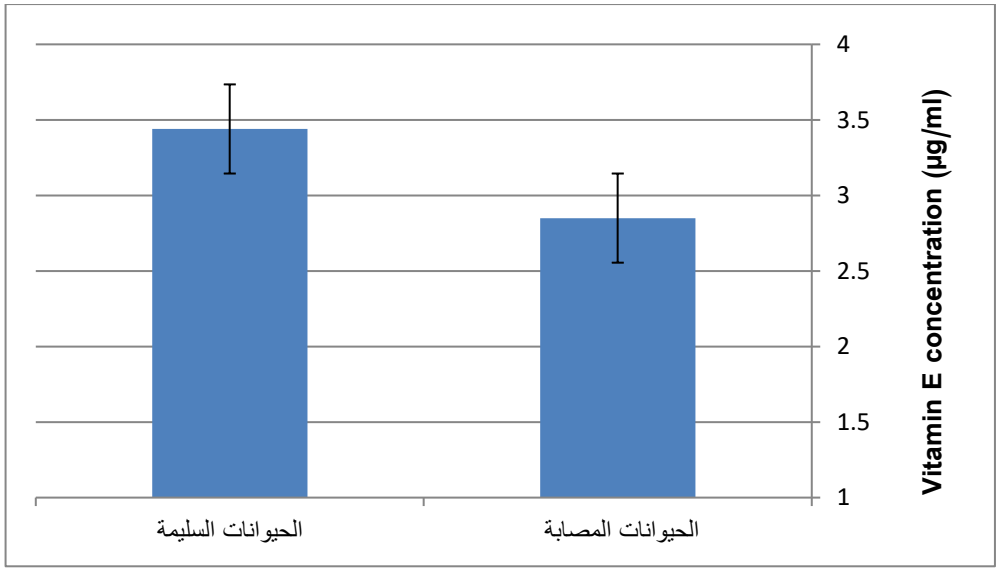

Figure (2): Vitamin E concentration in healthy and infertile ewes The values are expressed as standard error ± average.

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