Multidisciplinary

Study of low-density lipoproteins extracted from egg yolk, and proline on physiological effect in frozen semen parameters for Awassi sheep

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Abstract
The study aimed to determine the optimal concentration of the amino acid proline and low-density lipoproteins (LDL) extracted from egg yolks to be used individually or synergistically in dilution solutions, and to estimate their effectiveness in preserving the dilated and frozen semen inside and outside the reproductive septum of Awassi rams and to determine its fertilizing capacity.
It was concluded that the use of a dilution solution containing together LDL molecules at a concentration of 8% and proline at a concentration of 25% mmol can be used as an alternative to the imported Andromed solution in diluting the semen of Awassi rams, freezing and preserving it for long-term periods, and insemination with it to obtain good birth rates.

Keywords: awassi sheep, semen, low density lipoproteins, proline, artificial insemination

1. Introduction

Awassi sheep are the most important component of livestock in Syria, as their numbers exceeded 28 million heads (Group Agricultural Statistics, 2002), and constituted about 53.04% of the total main animal units (cows, sheep, poultry, goats, buffaloes) that produce food, and contributed to providing about 63.5% of meat. The locally produced red meat was followed by cows, buffaloes, and goats, with rates of 35.6, 25.6, and 3.5%, respectively. It also comes in second place after cows in milk production, with a rate of 08.5% of the total quantities of milk Produced.

It has a comparative advantage in the production and fattening of sheep so that an export surplus of more than 0 million can be found. annual wages without the local market being affected and maintaining its balance (Aurich, 2005) [1], which adds to it. Its strategic importance makes it an important source of animal protein that can contribute to reducing the nutritional gap. It also gives it an important Arab dimension, especially since the imports of Arab countries to cover the food gap exceeded 35 percent. billion US dollars in 2010 (Arab Organization for Agricultural Development, 2010), then the Arab food deficit. It is expected to increase with the continuation of the high population growth rate, estimated at 0.2% in the Arab countries in parallel with the depletion of natural resources.

In view of the importance of the Awassi sheep and their great role in the production process, it was necessary to pay attention to their care and breeding in order to improve. Its productive and reproductive capabilities, being from the local races settled in Syria and neighboring countries for thousands of years, it has acquired, whether by natural or directed selection, the ability to adapt and produce in harsh environmental conditions. I am still often subject, in the systems of her care, to the theatrical systems that spread over large areas of the valleys with an atmosphere Harsh climatic conditions compared to the environments in which other internationally famous sheep breeds, such as Allest-Friesian producers, live. For milk and Suffolk meat-producing and many more. Which made it gain an additional advantage because its breeding takes place in different regions

Environmental that does not allow the practice of any form of plant or even animal production on the other hand, the improvement of their productive and reproductive indicators as a result of the long genetic improvement operations carried out. In the breeding stations of the Ministry of Agriculture and Agrarian Reform, the possession of Awassi sheep showed hereditary capabilities. latent that encouraged the continuation of genetic improvement plans and the trend towards the use of reproductive biotechnologies such as vaccination the transfer of embryos to accelerate the dissemination of genetic factors characteristic of elite animals on a large scale among. The herds of small breeders locally and in the Arab world (Akhter, 2011) [2].

2. The importance of Awassi sheep and their characteristics

2.1 Awassi sheep is the only race in Syria and the most widespread in the Middle East and West Asia. it has been raised in the Arab region for at least five thousand years and belongs to the thick-tailed or tailed wool veins and is famous for its multiple production capacities.

ICARDA, 2003; highly adaptable to multiple ecosystems and walking great distances 0222), which helped spread it in a number of Arab countries and some Asian countries, African and European all the way to New Zealand and Australia. It is believed that its name came after to one of the Arab tribes that cared for its cultivation in the past between the Tigris and Euphrates rivers and in Turkey it is called Arabic.

Fig 1: A head of a sheep from the Uruk region in southern Iraq, dating back to the third millennium BC (3000-3300 BC) (British Museum)

2.2 Reproductive characteristics of Awassi sheep

Although Awassi sheep are among the breeds that produce good milk their efficiency that reasons for the decline in the birth rate of Awassi sheep are due to the tendency of breeders towards selection in favor of (Epstein) milk production and large body size and neglect of reproductive efficiency, as was the case with Al-Aroy. The decline indicates that the traits of livability in harsh environmental conditions are more likely than the traits of high productivity.

There are many reasons for the remarkable variation in the fertility of sheep flocks, and this is due to various reasons, including administrative (the way of feeding, and the nature of pastures), including environmental (different conditions surrounding the animal), in addition to the animal's health and physical condition before, during and after the breeding season, as well as diseases.

2.3 Artificial insemination

The productivity and reproductive indicators of sheep can be improved by various methods, such as selection, cross-breeding, and others. The use of artificial insemination is the most effective way to accelerate the genetic improvement of farm animals (Kubovičová, 2011) [13].

2.3.1 The development of semen handling methods

The accumulation and development of human knowledge in various fields of life sciences, especially at the cell level (cell physics, cell chemistry, cell physiology) has contributed to the development of semen handling technologies, especially after understanding the role of the different components of semen extension solutions in protecting sperm from the resulting effects. On the processes of freezing and thawing and providing the "ideal" environment to continue life after exposure to thermal stresses and damages resulting from the processes of freezing and thawing. Semen dilution solutions play an important role in the success or failure of the artificial insemination process. It is known that the dilution solution does not increase the sperm fertility, but rather maintains its vitality and prolongs its life. Fertilization and storage of semen. The dilution solution is used to increase the volume of the ejaculate to obtain several doses of which are transportable and circulating to be used in the artificial insemination of several females, provided that this solution preserves the integrity of the sperm and its functional characteristics and gives appropriate fertilization rates or close to those obtained by natural insemination.

The semen is used for artificial insemination either fresh or chilled to + 5 °C or frozen (after thawing it with liquid nitrogen at a temperature of -246 °C, where cryopreservation is a widely used technology for the long-term preservation of semen (Purdy, 2006) [20]. In order to freeze the semen, it is cooled after dilution to a temperature close to zero Celsius, and then kept at that temperature for a period of time with the aim of equilibrium and cooling, which is the period of time required to reduce the rate of metabolism and ensure the penetration of glycerol into the sperm to achieve balance in the concentration of glycerol. And the rest of the components of the dilution solution affecting the urethral pressure of the medium, and this period ranges between 1-3 hours (Salamon Maxwell, 2000) [22]. Decuadro-Hansen (2004) stated that cooling the semen to a temperature of +5 °C before freezing is necessary to protect sperm Of the negative effects of the land shock, however, the rapid implementation of this process to +10 °C led to a decrease in the movement of frozen ram semen after thawing, Fairfull and Fiser (2486), compared to the traditional cooling process that includes the incubation of straws filled with semen in a cold at a temperature of + 5 ° C and within 2-3 hours.

Kumar (2009) [12] found that the implementation of the cooling process automatically at a rate of 0.15 h / min using a programmed freezer improved the possibility of freezing the sperm of Indian rams, and the observed superiority after thawing in the tested indicators (motility, integrity of the terminal body) compared to the traditional method. Ashrafi (2011) reached the same conclusion when automatically cooling the dilated semen of the Iranian Chal rams by means of an automatic cooler at a cooling rate of 0.30 h/min to reach 5 °C and then keeping it at this temperature for 80 minutes before freezing compared to traditional cooling for 24 hours. 3 hours before freezing, and recently through electron microscopy observations of rams' sperm at the end of the cooling stage, Demir (2015) [6] found that increasing the cooling rate to 0.6-0.9 h/min led to an increase in the percentage of damage to the morphological structure of the sperm due to the decrease in temperature. This damage was concentrated particularly in the outer acrosome membrane and the plasma membrane of the sperm, and this negative effect also extended to the stage after freezing and thawing through the decrease in the sperm motility index that treated with a cooling rate greater than 0.3 h / min.

2.4 Basic components of semen extension solutions

Scientists were able to develop various dilution solutions and use cold protectors and multiple additives such as amino acids and antioxidants, in addition to developing methods of freezing and thawing as a result of technological progress and the use of modern laboratory equipment with a high degree of accuracy and efficiency and the use of appropriate inoculation tools. Several dilution solutions were used to preserve the semen of rams, whether for short-term or long-term preservation. The composition of the dilution solution usually differs from one country to another, and even from one laboratory to another in the same country (Marques, 2006) [17]. To determine the efficiency of the dilution solution, the percentage of live sperm was adopted after Thawed in addition to the fertility rate of females inseminated with frozen semen as two main criteria.

It was pointed out that the main objective of using dilution solutions is to protect the sperm from the lethal effects of the formation of ice crystals during freezing, and to reduce the amount of damage they are exposed to during the freezing and thawing processes (Amirat, 2007) [3], as the components of the dilution solution affect the survival rate of sperm Frozen after thawing (Curry, 2000, Leibo, 2002) [5, 11]. Despite the many types of dilution solutions used, the ingredients included in the composition of any of them must provide the following:

2.4.1 Source of energy

Sperm need a food source to maintain its cellular activity, and are simple sugars (fructose).and glucose (contained in the seminal plasma or within the dilution solutions, the main nutritional source for the sperm, as was the use of sucrose, sorbitol, and pyruvate (Vishwanath, 2000) [25].

2.4.2 Buffering capacity

The dilution of the semen in a solution that has a good ability to regulate the pH of the medium is important to maintain the vitality of the sperm during the freezing and thawing processes (Rasul, 2000) [21]. Where the decrease in the pH value results from the accumulation of lactic acid as one of the by-products of the metabolic processes carried out by the sperm to secure the energy needed for its movement. Raise the pH within a narrow range between 6.5-7.5 (Salamon, 2000) [22] To curb changes in the pH of the semen during storage and preservation, substances that have the ability to regulate the pH are added to the dilution solution and are known as protective or buffer materials, which are With a weak acid or a weak base added to water to form a protective (buffering) solution, it prevents significant changes in the pH when adding small or medium amounts of a highly acidic or alkaline substance to the solution, that is, it neutralizes the medium. Thus, it maintains the degree of the acidity of the solution pH and diluted semen close to a constant value (Harris, 2003) [8].

2.4.3 Controlling the bacterial growth

The International Organization for Animal Health recommended adding antibiotics to dilution solutions used to freeze semen to prevent bacterial infection (OIE, 2008). Almquist (1948) found that adding 500 to 1000 IU/ml semen improved the fertility of semen. For low-fertility heifers, Bratton and Foote (1950) also found a significant increase in the non-estrus rate of cows inseminated with semen supplemented with 1000 IU of penicillin or/and 1000 µg of streptomycin per ml dilution solution. Maxwell and Salamon (2000) [22] by adding 1000 international units of penicillin and 1 mg of streptomycin together per 1 ml of ram semen extension solution, and in addition to the effectiveness of these two compounds in maintaining the characteristics of rams semen dilatation, Ismaeel and Azawi (2012) found that the addition of cephalosporins at a rate of 1 mg / ml Extension solution was more effective in inhibiting bacterial growth and improving the dilated semen characteristics of Awassi rams.

2.4.4 Cool and cryoprotectants

The type and concentration of cooling and antifreeze protectors used in dilution solutions are among the factors affecting the success of freezing ram semen (Nur, 2005) [18] and preserving its fertile capacity (Wulster-Radcliffe, 2004) [24], as it works to protect sperm from the negative effects of cold shock during the two cooling processes Freezing and thawing, mainly using egg yolks or boiled, skimmed or full-fat milk, as substances that are useful not only as a good food source, but also in protecting sperm from cold and heat shocks due to their richness in lipoproteins and lecithin.

2.4.4.1 Using egg yolk in dilution solutions

The use of egg yolk in semen extension solutions dates back to the beginning of the last century, when Phillips discovered in 1939 its ability to protect bull sperm from the effect of low temperatures when used in solutions prepared for preserving bull semen. Now, the egg yolk is a commonly used material in semen extension solutions prepared for freezing. Around the world, it has been used at a concentration of 20%. Muino (2007) [16] indicated that the protective action of the egg yolk from the effect of cold shock comes through its ability to adhere to the plasma membranes of the sperm during the freezing and thawing processes, due to its richness in phospholipids, cholesterol and low-density lipoproteins that maintain the integrity of the membrane. Plasma of sperm from the effect of cold shock during refrigeration, freezing and thawing (Hu, 2010, Aurich (2005) [10, 1] mentioned that the contents of the egg yolk of these components preserved the integrity of the phospholipids of the plasma membrane of horse sperm during cryopreservation. It was found that the use of egg yolk A number of disadvantages, as it is described as having a very complex chemical composition, as it contains about 300 types of different proteins.

2.4.4.2 Low density lipoproteins

They are particles with a spherical shape, with a diameter between 20 and 60 nanometers, with a density of 0.982 g / ml (Anton, 2007) [3] consisting of a core of lipids (triglycerides and esters of cholesterol) surrounded by a monolayer of phospholipids and proteins that are distributed within this layer and are called Then, with apoproteons, the LDL molecule consists of 11-17% proteins and 83-89% lipids. Phospholipids

represent 26% of the total lipids included in the structure of the LDL molecule. They play an important role in the stability and stability of the LDL structure. Phosphatidylcholine forms the section The largest of the phospholipids included in the structure of LDL, and the distribution of cholesterol within the phospholipid layer contributes to increasing the rigidity and stability of this structure (Anton, 2007a) [4].

2.4.4.3 Cryoprotectant

The antifreeze protectors used in dilution solutions are classified into two main categories according to their ability to penetrate through the plasma membrane of the sperm and cross into the cellular medium. They are penetrating cryoprotectants and non-penetrating cryoprotectants (Purdy, 2006) [20].

Since the English scientist Polge discovered in 1949 the protective effect against freezing that glycerol possesses, it quickly became one of the most famous and widely used antifreeze protectors in freezing the semen of thorns and other animal species (Foote, 2002) [7]. (Holt, 2000) [9] and DMSO dimethyl sulphoxide, which was successfully used to freeze elephant semen. Currently, glycerol is one of the most widely used antifreeze solutions in ram semen dilution solutions, and is characterized by its ability to penetrate through the sperm plasma membrane. It was noted that glycerol crossed into the sperm of horses one minute after it was added to the medium in which it was preserved, as it binds to water molecules by hydrogen bonds (Palacin, I., J.A, 2008) [19], thus preserving cellular water within the sperm, which is a process necessary to maintain the size and structure of cells And protecting it from rupture when the water freezes, as glycerol interacts with ions and macromolecules in the cellular medium, and works to reduce the freezing point of water, which leads to a decrease in the electrolyte concentration in the remaining non-freezing part of the cellular water and thus a decrease in the ice crystals formed inside the sperm (Medeiros, 2002) [15] and the ideal concentration of glycerol within the dilution solutions ranges between 6-8%, as its concentration is related to several factors, including the rates of cooling and freezing used, the composition of the dilution solution, and the method of adding glycerol to the samples (Maxwell and Salamon, 2000) [22].

3. Recommendations

Studying the effect of using LDL molecules alone or with other amino acids or other additives such as vitamins and antioxidants in semen extension solutions for other animal species and investigating the levels of the hormone oxytocin in the blood of sheep during the estrus period in order to try to overcome its negative effect recorded on fertility indicators in a way that facilitates Performing the artificial insemination process deep in the uterus.

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