تأثير محاليل تمديد مختلفة في خصوبة السائل المنوي المجمد لكباش العواس

Effect of Different Extenders on Awassi Rams Frozen Semen Fertility

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الملخّص

نُفذ البحث في محطة بحوث ازرع، التابعة للمركز العربي لدراسات المناطق الجافة والأراضي القاحلة/أكساد، خلال الموسم التناسلي 2014، بهدف تقييم القدرة الإخصابية للسائل المنوي المجمد لكباش العواس في ثلاثة محاليل تمديد، ولاختبار فاعلية المعاملة بهرمون الأوكسيتوسين قبل تنفيذ التلقيح الاصطناعي في عنق الرحم.

قُسمت 90 نعجة عواس عشوائياً إلى ثلاث مجموعات رئيسة متساوية لتقييم تأثير محلول التمديد في جودة السائل المنوي، وقُسمت المجموعة الرئيسة إلى تحت مجموعتين فرعيتين متساويتين لاختبار أثر الحقن بالأوكسيتوسين. لُقحت الإناث الشبقة، التي تزامن شبقها باستخدام اسفنجات مهبلية، لكل مجموعة رئيسة بسائل منوي مُجمد بأحد محاليل التمديد التالية: محلول قياسي (AndroMed®)، ومحلول صفار البيض (20 %) مع الحمض الأميني برولين (25 ميلي مول/مل) (EY+25P)، ومحلول ليبوبروتينات منخفضة الكثافة (8 %) مع البرولين (25 ميلي مول/مل) (LDL+25P). وحُقنت نعاج إحدى تحت المجموعتين لكل مجموعة رئيسة وريدياً بجرعة 20 وحدة دولية من الأوكسيتوسين قبل التلقيح بنحو 10 دقائق.

أظهرت النتائج تأثيراً ايجابياً لمحلول (LDL+25P) في مُعدلي الإخصاب الظاهري والولادات (62.06±0.93، و41.38±0.91 %، على التوالي) مقارنة بالمحلولين الآخرين، إذ بلغ المعدلان (56.67±0.91، و 40±0.90 %) و (48.28±0.93، و 31.03±0.91 %) في مجموعتي محلول (AndroMed®) ومحلول (EY+25P)، على التوالي.

كما انخفض مُعدّلي الاخصاب الظاهري والولادات نتيجة المعاملة بالأوكسيتوسين من 65.12±0.76، و 44.19±0.75 % على التوالي في النعاج غير المعاملة بالأوكسيتوسين إلى 46.67±0.74، و 31.12±0.73 % على التوالي في النعاج المعاملة به.

يستنتج أن استخدام LDL والبرولين في محاليل تمديد وتجميد السائل المنوي لكباش العواس حَسّن معنوياً مُعدلي الإخصاب الظاهري والولادات، في حين كان تأثير المعاملة بهرمون الاوكسيتوسين سلبياً في المؤشرين.

الكلمات المفتاحية : الليبوبروتينات منخفضة الكثافة، الأوكسيتوسين، البرولين، محاليل تمديد، التلقيح الاصطناعي، كباش العواس.

Abstract

The research was carried out at Izra'a research station (Arab Center for the Studies of Arid Zones and Dry lands / ACSAD) during the mating season of 2014 to evaluate the fertilizing ability of Awassi ram's frozen semen diluted in three extenders, and to test the oxytocin treatment effectiveness before cervical artificial insemination (CAI).

Ninety Awassi ewes were randomly divided into three equal main-groups to test the extender impact on semen quality;

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each main group was divided into two equal subgroups to test the oxytocin impact. Ewes in synchronized-estrus (through vaginal sponges) of each main group were inseminated using frozen-thawed semen diluted in one of the following extenders: standard (AndroMed®), egg yolk (20%) adds 25 mM/ml proline (EY+25P), and low-density lipoprotein (8%) adds 25 mM/ml proline (LDL+25P) solutions. Ewes of three subgroups were intravenous injected with oxytocin (20 IU) 10 minutes before CAI.

Results showed positive effect of (LDL+25P) extender in fertilization and lambing rates (62.06±0.93 and 41.38±0.91 %, respectively) compared with two extenders, where (56.67±0.91 and 40±0.90 %) and (48.28±0.93 and 31.03±0.91 %) recorded, respectively in (AndroMed®) and (EY+25P) extender-groups.

Fertilization and lambing rates were negatively influenced by oxytocin treatment, they decreased from 65.12±0.76 and 44.19±0.75 % in non-treated ewes to 46.67±0.74 and 31.12±0.73 % in treated ewes, respectively.

In conclusion, the use of LDL fractions with proline increased significantly fertilization and lambing rates, while the effect of oxytocin treatment was negative in both traits.

Keywords: Low-density lipoprotein, Oxytocin, proline, Semen extender, Artificial insemination, Awassi rams.

Introduction

The use of frozen semen in an artificial insemination of sheep still limited (Holt, 2000) because of the difficult anatomical nature of the cervix, which acts as a barrier, and prevent the semen straw to reach the uterus body (Curry, 2000).

In only 2% of the ewes might the catheter (Straw) of artificial insemination naturally deliver the semen deep within the cervix or been by passed directly to the uterus (King et *al*., 2004), where cervical rings interfere with each other irregularly, and the amount of expansion and openness does not exceed 3 mm during the period of estrus in non-pregnant ewes (Halbert *et al*., 1990). So, the experts of artificial insemination deposit semen in the cervix, which is known as cervical insemination, and requires the use of a relatively high number of sperms in the dose (Colas, 1983). However, the ratio of fertilized ewes remains relatively low after an artificial insemination in the cervix with the frozen semen (Yoshida, 2000), as fertility rates rarely exceed 40% (Salamon and Maxwell, 1995a), and in some field studies a fertility rate of less than 20% was recorded (Windsor, 1997).

Relatively low fertility rates after the artificial insemination with frozen semen in sheep are not only resulted by the anatomy nature of the cervix, but also from the irreversible damage, which occurs to sperms during the processes of freezing and thawing (Bailey *et al*., 2000; Salamon and Maxwell, 1995b). Despite of the high percentage of live sperms ram after thawing (40-60%), it was observed that not more than only 20-30% of the sperms were not damaged in terms of vitality and retained the ability to fertilize (Salamon and Maxwell, 2000), which indicates that the components of the semen extender might help in susceptibility of semen to fertilize ova after freezing and thawing (Salamon and Maxwell, 1995b).

Egg yolk is widely used as a main component of semen extenders, it works as buffer which protect sperm from the effect of cold shock during the freezing and thawing processes (Luster, 2004). Because it contains the low-density lipoproteins (LDL) particles which form a thin film on sperms protecting from the damage caused by crystallization during the freezing conservation (Moussa et *al*., 2002).

Some amino acids (glutamine, proline, histidine, glycine) have been added to the semen extenders to protect sperms at freezing of semen ram (Sanchez-Partida et al., 1992; Sanchez-Partida et *al*., 1998), semen horse (Trimeche et *al*., 1999), semen's buck (Al Ahmad et *al*., 2008), and bull semen (Amirat et *al*., 2009). Scanchez-Partida et *al*., (1992) found that the use of glycine betaine (tonic for lipid metabolism), and proline in low concentrations caused improving mobility of sperm ram after freezing and thawing in presence of glycerol and egg yolk.

On the other hand, the artificial insemination in sheep could be performed in different ways resulting different pregnancy rates in accordance to the deposit place of semen in the genital tract of the female. Fair et *al*., (2005) has stated that the deposit place of frozen thawed semen mainly affects the fertilization rate among ewes artificially inseminated.

Several methods have been used to improve fertilization rates in ewes when conducting the artificial insemination using frozen semen by trying to deposit semen deeper as possible in the cervix. Kaabi et *al*., (2006) found a positive correlation

between the depth of semen deposit in the cervix and the fertility by implementing cervical artificial insemination.
The increase in the depth of frozen semen deposit inside the cervix contributed to high lambing rate from 18.2% when semen was directly deposited in the vagina to 76.4% when semen was deposited at a depth of more than 4 cm inside the cervix (Salamon and Maxwell, 2000). The deposition of frozen thawed semen directly in the uterine horn gave in some studies high lambing rates, which ranged between 50% to 80% (Youngquist and Threlfall, 2007).
To achieve this goal several attempts were carried out, as Varnavskij and Turbin (1974) tried to increase the systolic activity of the cervical muscle through electric stimulation (3.5 volts), but this method doesn't have any positive impact on the lambing rate. Cappai et *al*., (1998) had the ability to deliver the semen in a depth of 2-3 cm inside the cervix or immediately in the uterus after withdrawal of the cervix opening into the vagina entrance using surgical tweezers. Andersen et *al*., (1973) pointed out that this method has improved the fertility rate, however, maligned by increasing the stress on the animals.
Several drug treatments were also used like injecting ewes with hormones as oxytocin or prostaglandins (PGE_1, PGE_2, $PGF_{2\alpha}$) before the implementation of artificial insemination. To achieve relaxation and expansion of the cervix and to increase systolic activity of the cervix and uterus, which improved the sperm mobility into the genital tract of the female (Salamon and Maxwell, 2000). Khalifa et *al*., (1992) was able to overcome the cervical rings and to deliver the semen for a distance of 6.1 cm inside the cervix when ewes were intravenous injected with 400 USP-unit of the oxytocin, while the distance of semen deposit did not exceed 2.9 cm inside the cervix in the ewes group, which artificially inseminated without oxytocin treatment.
This research aims to test the fertilizing ability of frozen semen collected from Awassi rams and diluted using an extender containing low-density lipoprotein (LDL) fractions adds proline in comparison to other two used extenders, and to test the effect of oxytocin treatment before conducting the artificial insemination on some fertility parameters in Syrian Awassi ewes.

Material and methods

1. Animals:

Three rams of the Awassi sheep breed at 3 years old, and an average live weight of 75 ± 3 kg were used in this study. They were raised in Izra'a research station (Dara'a province/Syria) which belong to the Arab Center for the Studies of Arid Zones and Dry lands (ACSAD) and were used to collect semen during the mating season of the year 2014. As well as 90 ewes of Awassi sheep breed, at ages ranged between 2 to 5 years and with an average live weight of 61.4 ± 1.3 kg were also used for this study. All experimental animals in the separated males or females flocks underwent the same feeding and housing conditions, and were given flushing diet for 15 days before the start of mating season. Females were randomly distributed into three equal main groups (n = 30), convergent in age composition and average live weight, to test the effect of 3 type of extenders.
Each main group was divided into two equal subgroups randomly (n = 15) which were also similar in age and live weight. One subgroup from each main group was allocated to an additional hormone treatment represented in one oxytocin injection; the other subgroup of each main group remained without this hormone injections in order to test the effect of this treatment before implementing the artificial insemination on the studied traits.

2. Extenders:

Three types of extenders (Table 1) were used as follows:
- Standard extender (AndroMed®) which is a commercial prepared solution, and was adopted as control.
- A locally prepared extender containing egg yolk (EY) in concentration of 20% (v/v), as well as amino acid proline (P) in concentration of 25 mM / ml (Millimole/milliliter); symbolized by (EY+25P).
- A locally prepared extender containing low-density lipoprotein (LDL) in concentration of 8% (w/v) as well as 25 mM proline / ml; symbolized by (LDL+25P).

Table 1. Basic components of extenders used to dilute and freeze the semen ram's.

	Types of used extenders		
Basic Components	Standard (AndroMed®)	20% egg yolk + 25 mM proline (EY+25P)	8% LDL + 25 mM Proline (LDL+25P)
	Not Clarified	3.52 g Sodium Citrate	
		20% Egg yolk (v/v)	8% Low-Density Lipoprotein (w/v)
		25 mM Proline / 1 ml Solution	
		194 mg Glucose	
		6.4% Glycerol	
	4 Antibiotics	Streptomycin 100 mg/ml, and Penicillin G 100 IU/ml	
	Bi-Distilled Water up to 100 ml		

Low-Density Lipoprotein (LDL), Proline (P), Egg Yolk (EY).

3. Semen handling:

The preparation of frozen semen straws was carried out in the laboratory of artificial insemination and embryo transfer in Izra'a research station ACSAD in the same mating season according to the following procedures:

After double sequentially collection of the semen from each ram using an artificial vagina, the semen quality was evaluated for each male in terms of color, volume, and density. A phase contrast microscope (Nikon Eclipes e400, Japan) was used to evaluate the motility of sperms and their use ability.

The collected semen from each ram was divided into three parts using a micropipette, each part was diluted in one of the three mentioned types of extender (Table 1). After cooling and equilibration period, the diluted semen was filled in 0.5 cc straws, each contained about 250 × 106 sperms. A programmed freezing system was used (Digitcool, IMV Technologies) to decrease the straw temperature quickly from +4°C (cooling temperature) to -140°C during 4 minutes in the liquid nitrogen vapor, thus leads to quickly freeze of semen within the straws which have been then cryo-preserved in liquid nitrogen (-196°C) until their use for insemination.

The thawing of the cryo-preserved semen occurred through immersing the frozen straw in a water bath at +37°C for 30 seconds (Nur et *al*., 2011). The straw was dried with a tissue before inserting it inside the inseminator to implement the artificial insemination.

4. Estrus synchronization:

The timing of estrus appearance was reached using vaginal sponges saturated with Fluorogestone Acetate (FGA-40 mg), which remained in the vagina for 14 days according to the manufacturer's directions. Simultaneous to the withdraw of vaginal sponge, each female was injected intramuscularly with 500 IU of pregnant mare serum gonadotropin (PMSG/eCG) (Zarkawi, 2010). Teaser males were released to the female groups after 24, 36 and 48 hours from sponge withdraw to detect the females in heat that responded to the estrus synchronization treatment, which have been isolated in a special sector to be artificially inseminated.

5. Treatment with oxytocin:

Half number of the total females (n = 45 from 3 subgroups, each 15 ewes) was devoted to test the effect of oxytocin injecting before the artificial insemination on fertilization, and lambing rates. The injection was carried out in the jugular vein at a dose of 20 IU of oxytocin per female in the related subgroups and was done 10 minutes prior to the implementation of cervical artificial insemination.

6- Implementation of the artificial insemination:

Ewes in estrus of the first main group (control group) were inseminated with frozen semen which were extended using the standard solution (AndroMed®), half number of ewes in this group (one subgroup) were injected with oxytocin, while the other half (second subgroup) of ewes were also artificially inseminated using the same extender (AndroMed®) but without oxytocin injection. The ewes in heat at the second main group were artificially inseminated with frozen semen extended using the (EY+25P) solution, where the half number of ewes undergo the injection of oxytocin and the other half had not been injected. The same procedure applies to the third main group which were also artificially inseminated with frozen semen extended using the (LDL+25P) solution.

The artificial insemination technique used in this study was the cervical method (passing an inseminator containing a straw into the cervix deep as possible) using one straw containing about 250 million sperms. The implementation of the insemination was carried out 55 hours after sponge withdrawal trying to reach accuracy in insemination time for all females and to organize the exact time of applying the oxytocin injection. The procedure of the insemination includes entrance of each individual ewe to the insemination in accordance with the sequence of the sponge withdrawal as well as oxytocin injection, where the hind limbs of the ewe were lifted to a wooden locally manufactured stand. The ewe was fixed by workers to ensure calmness during the process. A vaginal speculum handle with illumination have been inserted into the vagina to determine the cervix opening accurately. Then, the inseminator (insemination gun) which contains the post-thawed semen straw, was introduced through the vaginal speculum to the cervix opening where the inseminator was slowly pushed inside the cervix for a distance of 1 to 3 cm or more as deep as possible. The semen was slowly pushed through the inseminator to ensure an internal deposit of the sperms in the deepest possible point within the cervix.

7. Fertilization, and lambing follow-up:

After 17 days of artificial insemination teaser rams were introduced to the inseminated ewes to detect return estrus and calculate the non-return rate (fertilization rate) for each group. After 50 days of insemination a pregnancy test for all fertilized female was carried out using real-time ultrasonic scan device (Honda,madein, Japan 2000) to detect pertlizatios with high accuracy at a relatively early time of pregnancy and to calculate the pregnancy rate (number of pregnant females diagnosed using ultrasound / number of females inseminated x 100). After lambing time the lambing rate was recorded (number of lambed and aborted ewes / number of ewes inseminated x 100).

8. Statistical analysis:

Data were analyzied using Factorial Design 3×2 with treatment with/without oxytocine, extender type and their interactions using the General Linear Model (GLM) procedure of SAS (2008) as following linear model:

$$Y_{ijk} = \mu + E_i + F_j + (E \times F)_{ij} + e_{ijk}$$

Where:

Y_{ijk}: the studied variables.

μ: the grand mean.

E_i: the effect of extender, where i = 1 (AndroMed solution), i = 2 (sodium citrate solution with egg yolk and proline), i = 3 (sodium citrate solution with LDL and proline).

F_j: the effect of oxytocin treatment, where j = 1 (treatment with oxytocin), j = 2 (no treatment with oxytocin).

e_{ijk}: random error (residual), which is assumed to be a normal distribution with mean of zero and variance $I\sigma^2$.

The percentage values of fertilization rate, and Lambing rate were edited to exponential transformation to perform the analysis of variance.

Duncan's multiple range tests (1955) was used for treatment comparisons of values that were found significant by ANOVA. The least squares mean is used to explain the significance of the differences.

Results and Discussion

Analysis of variance (Table 2) shows that the type of extender, oxytocin treatment and their interaction were high significantly affected (P<0.001) both fertilization rate (Non-return rate) and lambing rate. Here, it should be mentioned, that the pregnancy rates recorded in this experiment using ultrasonography method exactly matched the lambing rates in all groups, therefore the pregnancy rate will not be mentioned thereafter.

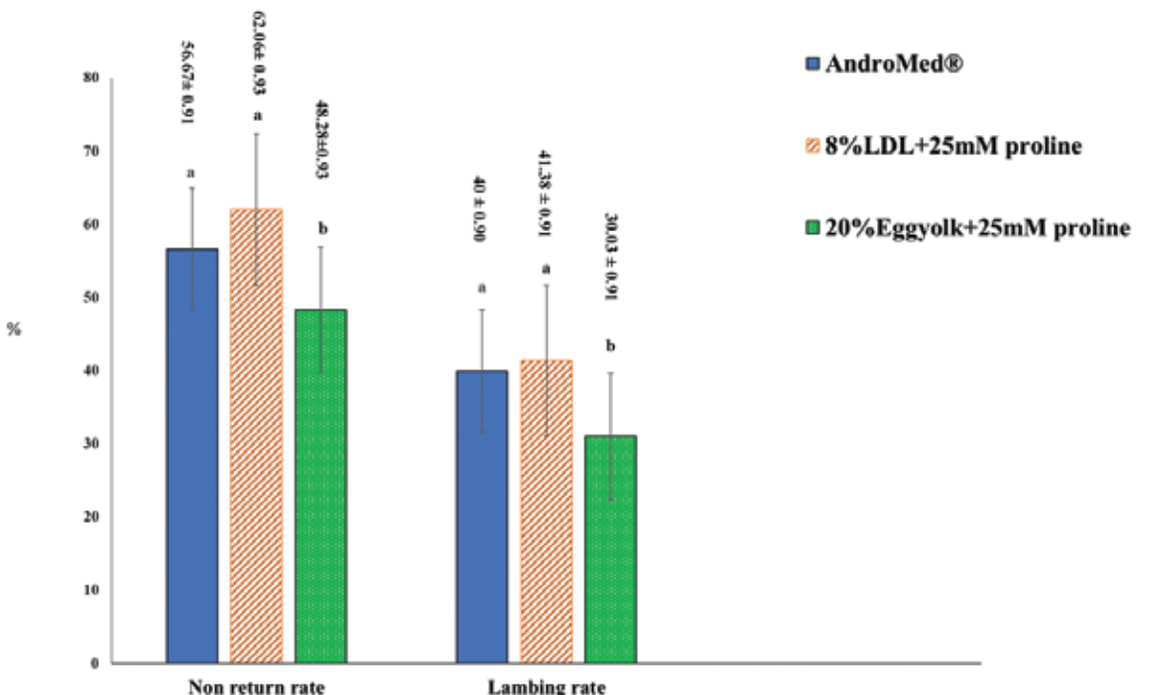
Table 2. Analysis of variance for the effect type of extender type, and treatment with oxytocin on the two studied reproductive parameters.

Source of variation	DF	Fertilization rate	Lambing rate
Extender	2	(1.33)***	(1.75)***
Oxytocin	1	(1.37)***	(1.57)***
Extender×Oxytocin	2	(1.33)***	(1.57)***
Residual	82	1.14	3.77

.significant at 0.1% level ***

The extender (LDL+25P) that contain low-density lipoprotein in concentration of 8% adds amino acid proline in concentration of 25 mM/ml showed superiority to the other two solutions, while the standard extender (AndroMed®) outweigh the extender (EY+25P) which contain full egg yolk in concentration of 20% and proline in concentration of 25 mM / ml.

Figure 1 represents the averages of the fertilization rates and lambing rates of the three main groups (extender type effect). Values were (62.06 ± 0.93%) and (41.38 ± 0.91%), respectively in the main ewes group (two subgroups) that inseminated with semen extended and frozen using the solution (LDL+25P), whereas these values were (56.67 ± 0.91 %) and (40 ± 0.90%), respectively in the other main group of ewes that were inseminated with semen extended and frozen using standard solution (AndroMed®). They also were (48.28 ± 0.93%) and (31.03 ± 0.91%), respectively in the last main group of ewes that were inseminated with semen extended and frozen using the solution (EY+25P) (figure 1).



Different letters (a, b) over the column indicate a significant difference (P<0.001) between averages.

Figure 1. Effect of extender type on fertilization rate and lambing rate.

These results are higher than that reached by Kumar and Naqvi (2014) where the lambing rate ranged was between 19.3 and 26.4% in Merino ewes which artificially inseminated (cervical method) with frozen thawed semen, and was also higher than that reached by Sanchez-Partida et *al*., (1999). The pregnancy rate was 18.1% as estimated using ultrasound in Merino ewes were cervical inseminated with frozen thawed semen that were diluted using both Tris and egg yolk in concentration of 15% and proline in concentration of 65 mM.

It should be noted that the lambing rate in the current study reached 50% in the subgroup ewes that had been inseminated with frozen post-thawed semen extended with (LDL+25P) without oxytocin treatment (Table 3).

Table 3 shows fertilization rates and lambing rates in all subgroups (interactions between Extender type and Oxytocin treatment).

The current results (Table 3) converge in some of the subgroups with the findings of Aral et *al*., (2010) in the Turkish Awassi ewes that were inseminated artificially with fresh semen using the cervical method. The lambing rate was 46.7%, which indicates that the tested extenders and the method of freezing used in the current experiment helped to reach results convergence results of insemination with fresh semen in Awassi sheep.

Table 3. Least square means (LSM) on fertilization rates (non-return rate) and lambing rates in the experimental subgroups.

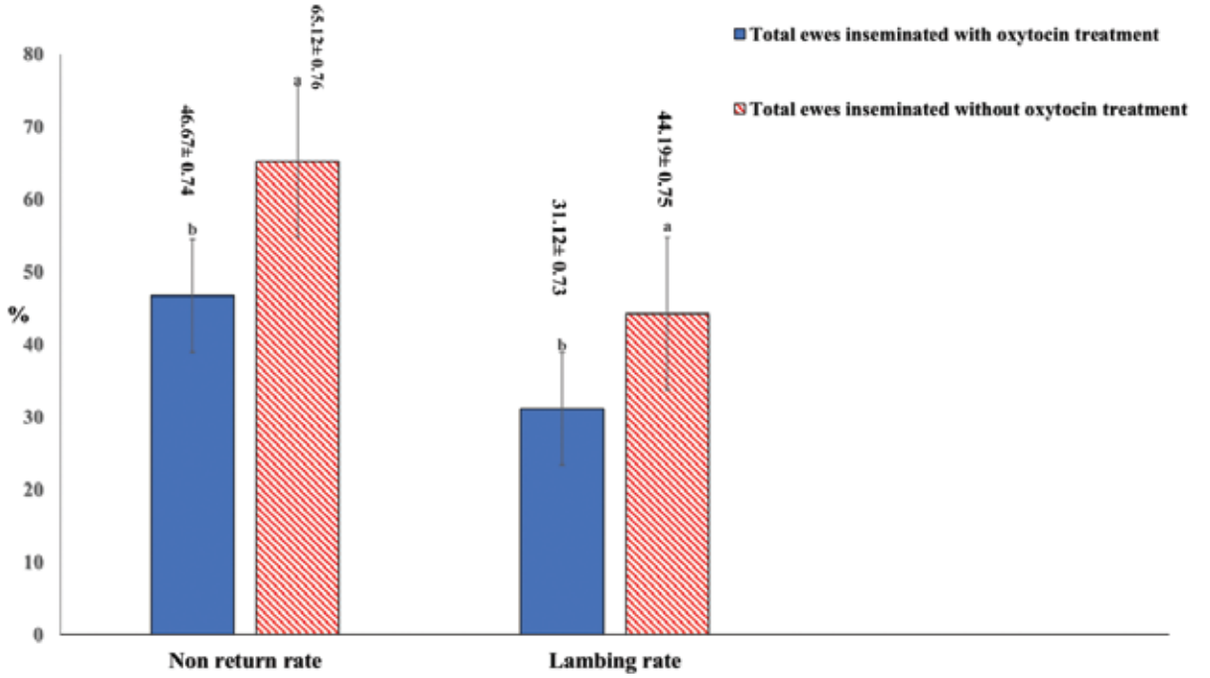
Groups Extender) (type	Subgroups: Extender, and oxytocin treatment	Number of ewes	Number of estrus ewes inseminated	Fertilization (%) rate	Lambing rate (%)
First (AndroMed®)	Standard extender AndroMed®; without oxytocin injection	15	15	66.67 ± 0.13^{a}	46.67 ± 0.19^{a}
	Standard extender AndroMed®; with oxytocin injection	15	15	46.67 ± 0.12^{b}	33.34 ± 0.17^{b}
Second (EY+25P)	Egg yolk extender (20%) ;adds 25 mM proline without oxytocin injection	15	14	57.14 ± 0.14^{b}	34.71 ± 0.16^{b}
	Egg yolk extender (20%) adds 25 mM proline; with oxytocin injection	15	15	40.0 ± 0.12^{c}	26.67 ± 0.17^{c}
Third (LDL+25P)	LDL extender (8%) adds 25 mM proline; without oxytocin injection	15	14	71.42 ± 0.14^{a}	50.0 ± 0.17^{a}
	LDL extender (8%) adds 25 mM proline; with oxytocin injection	15	15	53.34 ± 0.13^{b}	33.34 ± 0.15^{b}
(%) Total average				55.50 ± 0.18	37.50 ± 0.16

Low-Density Lipoprotein (LDL), Proline (P), Egg Yolk (EY). Different letters within the same column (a, b, c) indicate the presence of significant difference (P<0.001) between averages.

The technology used for the extraction and purification of LDL particles from the egg yolk, which had been developed by Moussa et *al*., (2002), may have contributed to the exclusion of sperm harmful components in the full egg yolk (Bencharif et *al*., 2008). Such granules, that have a negative impact on the frozen sperm ram (Watson and Martin, 1975), high density lipoprotein (HDL), and progesterone (Hu et *al*., 2011), in addition to calcium, which is available in high value in the egg yolk (approximately 133 mg/100g egg yolk) (Nys and Sauveur, 2004). Colas et *al*., (2009) has stated that in-vitro incubation of sperm ram in a media containing calcium has activated the capacitation stage of sperms

and stimulate the sperm acrosome reaction so that the ratio of sperms with intact membrane dropped to only 7.2% after entering the acrosome reaction stage. Therefore, the calcium is stimulating changes in sperm membrane of ram that lead to acrosome reaction entry (Grasa et *al*., 2006). The phenomena was resulting through cryo-preservation of sperms causing a failure in their ability to fertilize an ovum (Yanagimachi, 1994). Bashawat et al., (In Press). has pointed that LDL fractions in concentration of 8% adds proline in concentration of 25 mM/ml actually showed a synergistic act when used together in solutions to extend the semen of Awassi rams. This synergistic act contributed to the superiority of the total mobility and progressive motility indicators and the other mobility indicators of frozen post-thawed sperms which were estimated using CASA system (Computer-assisted sperm analysis). In a previous study Liu *et al*., (1991) indicate that the motility of sperms is the most important indicator in estimating the fertilizing capacity of the semen, and Januskauskas et *al*., (2003) confirmed the significant correlation between the motility indicators, assessed in-vitro using the CASA system, and the real fertility (*in-vivo*).

Concerning the carried out treatment with oxytocin, the results (Tables 2 and figure 2) showed, that the treatment with oxytocin was negatively affected (P<0.001) the studied fertility parameters (the fertilization rate and the lambing rate) that fell from 65.12±0.76, and 44.19±0.75%, respectively in ewes which artificially inseminated without oxytocin treatment to 46.67±0.74 and 31.12±0.73%, respectively in ewes which artificially inseminated after the oxytocin treatment (Figure 2). The lowest rates of fertilization and lambing (40.0±0.12 and 26.67±0.17%, respectively) have been recorded in ewes of the subgroup that were treated with oxytocin and artificially inseminated using egg yolk and proline (EY+25P) extender, while the highest value of the mentioned indicators (71.42±0.14 and 50±0.17%, respectively) have been registered in ewes of the subgroup that were artificially inseminated using (LDL+25P) extender without prior treatment with oxytocin (Table 3).



Different letters (a, b) over the column indicate a significant difference (P<0.001) between averages.

Figure 2. Effect of oxytocin treatment on fertilization rate and lambing rate.

These findings correspond with King et *al*., (2004) who found negative impact of the oxytocin treatment on fertility parameters using the Leicester hybrid ewes, where the lambing rate dropped from 40% in the control group to only 10% in the group treated with oxytocin (10 IU intramuscularly injection) 15-30 minutes prior to the cervical artificial insemination with frozen post-thawed semen containing 80 million sperms. King et *al*., (2004) interpreted the low lambing rate through the negative impact of oxytocin on sperm movement through the cervix in spite of its effectiveness in relaxing the cervix muscle and facilitating the implementation of artificial insemination through the cervix.

Similarly, Stellflug et *al*., (2001) found that the intravenous injection of ewes with oxytocin at a dose of 200 USP-Unit/ head half an hour before laparoscopic artificial insemination with frozen post-thawed semen have been negatively affected the fertility indicators.

In Stellflug et *al*., (2001) study lambing rate dropped from 63 % in the control group, which inseminated without oxytocin treatment, to 49.5% in the group treated with oxytocin. Stellflug et *al*., (2001) noted that the low fertility indicators after treatment with oxytocin could be caused by many factors that cannot be identified, especially as the short half-life of oxytocin, which does not help to determine the time and place for the negative effect of oxytocin in fertility indicators.

Conclusions and suggestions

- The in-vivo fertility of the frozen-thawed semen which has been extended using a locally prepared solution (extender) includes sodium citrate adds 8% low density lipoprotein (LDL) as well as the amino acid proline (25 mM/ml solution) excelled on those indicators resulting from the other two used extenders.
- It could abandon the import of the commercial extender AndroMed® and use a locally prepared extender includes LDL in concentration of 8%.
- It's not advised to use intravenous injection with oxytocin hormone for sheep before artificial insemination due to its negative impact on fertility indicators.

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