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Relationship between Age of Iraqi Awassi Ewes and Responses to Superovulation Protocols

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Abstract:

The current study aimed to investigate the relationship between the age of ewes subjected to various superovulation programs and the response of those ewes to the programs under study through the standard fertility parameters adopted in this type of studies.

Seventy healthy Awassi ewes were divided into seven equal groups (10 ewes for each) whose ages ranged from 2.5 to 7 years, with two age categories for each of the seven study groups, (the group of ewes less than 4 years old and the group of ewes aged 4 years and older) as follows: Control group (c) that did not receive any treatment (G1): a group of long-term progesterone sponges (14 days) with eCG injections on the day of the sponges' withdrawal, then GnRH injections on the day after the sponges were withdrawn. Group (G2) was the same as group (G1), but without GnRH injections. Group (G3) received short-acting progestin (CIDR) in the same design as (G1), while group (G4) received (CIDR) in the same design as group (G2), but for 7 days only days for both groups (G3, G4). While group (G5) was injected with PGF2α in two doses 9 days apart, the eCG hormone was injected one day before the second dose, then GnRH was injected one day after the second dose of PGF2. The last group (G6) is similar to group (G5), but without the GnRH injection.

The results recorded a superiority for ewes within the age category of 4 years and older in most of the study groups in the parameters of the twinning rate, and litter size rate, compared to the age category of less than 4 years, with the opposite in respect to litter size rate, when all groups recorded superiority for age category of 4 years and older with some exceptions. While no differences were recorded between the two age categories under study in the rest of the fertility parameters, namely the estrus rate,

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lambing rate and survival rate. The current study conclude that the best age for ewes subjected to superovulation programs should be confined Under 4 years old.

key words: Awassi, age, fertility parameters, Superovulation, ewes

Introduction:

The physiology, morphology, and hormone profile of the genital organs all undergo cycling changes during the estrous cycle, which is the rate at which female reproductive organs become functional following puberty (1). According to (2), the estrous cycle of Awassi ewes in Iraq lasted between 15 and 20 days, averaging 17.5 days from August to March. During the breeding season, a ewe's long phase of estrus can last anywhere from 20 to 36 hours, on average (3). The photoperiod, ewe age, and the presence of a ram in the flock all affect how long the estrous phase lasts. At the start or finish of the breeding season, the duration of estrous is shorter and can be as little as three to six hours (4). The ewe is a seasonally polyoestrous animal, meaning that it can only reproduce during a specific season and is sexually active during that time (5). The term "short-day breeders" refers to ewe that are exposed to short or falling day lengths, which induces oestrous, while long or increasing photoperiods commence seasonal anoestrous, or the non-breeding season (6). The degree of seasonality varies among breeds. According to (7), the majority of Syrian Awassi ewes breed between late June and early September, however the breeding season lasts from June to December (8). Because most of them reproduce seasonally, ewes are thought to have low reproductive efficiency because they have extended lambing intervals (9). Reproduction is the most economically significant characteristic in sheep production, and it is modifiable with hormone therapies (10). In order to coordinate estrus and promote conception, these hormonal therapies are employed in the breeding of sheep (11). Estrus synchronization is the reproductive process manipulated to allow sheep in a herd to accept mating and pregnancy at the same or near times of the year (12). The Optimal use of this technology in reproductive function enables us to tune in to estrus, increase the rate of ovulation, and increases the reproductive efficiency of agricultural animals (13). Prostaglandin (PGF2α), which is based on a system used solely during the breeding season, was used to synchronize estrus by ending the luteal phase by regression of the corpus luteum(14).

As not every stage of the estrous cycle responds to therapy in the same way, the procedure uses a double injection technique. In sheep, the LH peak surge occurs approximately 62–64 hours after injection, and the mean period from treatment to estrus occurrence was about 2 days (15). According to (16), ewes' estrus synchronization with vaginal sponges impregnated with flurogestone acetate

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(FGA) and eCG therapy is an efficient. Controlled Internal Drug Release (CIDR) Devices.

CIDR is T shaped apparatus which is safe to use on young ewes, easy to administer, inexpensive, and does not collect vaginal secretions or bacteria because it is made of rubber, which lowers the risk of vaginal infections and improves the technique of unifying estrus (17).

The present study was conducted to investigate the relationship between age of synchronized Awassi ewes and response to superovulation protocols by study different fertility parameters (especially rate of multiple births and litter size ate.

Materials and Methods

2.1. Experimental animals

The experimental protocol was completed in the Thi-Qar Governorate / Al-Shatrah District private lands. Seven groups of ten local breed ewes each, ranging in age from two to seven years, usedin this study. The investigation was prolonged from September 2023 to April 2024. Each group received three rams that were between two and four years old. To ensure their health and absence of illnesses, the animals underwent a thorough general and specialized clinical assessment. They also had a history of normal lambing. Prior to the study, the animals were identified by various colors.

2.2. Experimental design

A total of 70 ewes in 7 groups were included in this study, which employed 6 protocols (10 animals per group, ages 2 to 5 years) with a control group that received no treatment. The treatments were described as follows:

The control group (**c**) was left untreated.

Group 1 (**G1**): Using a specialized device, long-term progesterone intravaginal sponges (PIVS) were placed in the vagina.For fourteen days, the sponges were inside the vagina. After the sponge was withdrawn on day 14 and 500 IU were administered intramuscularly, GnRH was delivered one day after sponge withdrawal (WD).

Group 2 (**G2**): Use the same protocol as G1, excluding the injection of GnRH.

Group 3 (**G3**): 7-day estrus synchronization with short-term progesterone (CIDR) combined with an eCG injection during withdrawal (WD) and GnRH injection one day post-CIDR withdrawal:

Group 4 (**G4**): The technique is the same as in A, except GnRH is not used.

Group 5 (**G5**): Double dose (shoots) of PGf2α spaced nine days apart, eCG injection the day before the second dose, and a single dose of GnRH the day following the second dose of PGf2α are used to synchronize estrus.

Group 6 (**G6**): Use the same procedure as in G1, but omit the injection of GnRH.

2.3. Detection of the estrus

Animal reaction rate: A ram was used to observe ewes twice a day for an hour in order to detect estrous. On the second day after PMSG administration, estrous response rate a measure of the proportion of estrous ewes was determined by

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introducing an active fertile ram to the ewes under study. The data was then summed cumulatively to indicate the beginning of estrous.

3.4. Mating

A fertile rams would tend to estrous ewes. The evaluation of breeding soundness was conducted on the rams by several means, including semen analysis and a general clinical examination with a focus on the male gentle system.

3.5. Fertility parameters: Fertility parameters were determined using the following formulas in the study, in accordance with (18):

Twinning rate (%) = number of ewes giving birth to twins/ number of pregnant ewes × 100 **Litter size** = total number of lambs/number of ewes giving birth

Lambing rate (%) = number of ewes giving birth/number of pregnant ewes × 100

Estrus rate (%) = number of ewes showing estrus/number of ewes in the group × 100

Estrus onset = time between sponge withdrawal or the last injection and start of estrus

Conception ratio (%) = number of pregnant ewes/number of mating ewes × 100

Pregnancy rate (%) = number of pregnant ewes/number of ewes in the group × 100

Survival rate (%) = number of living lambs/number of lambs born × 100

In addition to calculate the **Gender of lambs also** determined at a birth time according to (19).

Lamb weight according to (20) when determined at the birth time by the electronic balance of single and twin lambs.

Statistical analysis

SPSS (Statistical Package for Social Sciences) version 31 for Windows was used to statistically analyze the gathered data. Percentages and statistics were used to define qualitative data. For acceptable comparisons between categorical variables, the chi-square test and Fisher's exact test were employed. According to (21), a P value of ≤0.05 was deemed statistically significant.

Results

Ewes aged 4 years and over showed superior rates of twin births compared to those aged less than 4 years, although the difference was not significant between the two age categories (Table1). Most of the groups subject to the study protocols recorded superiority for those aged less than four years in the parameters of conception rate and pregnancy rate (Figures1 and 2), with the opposite in respect to litter size rate, when all groups recorded superiority for age category of 4 years and older (except for G1 and G5) (Figures 3).

While the age of the ewes subject to the study did not show any effect on the rates of response to estrus to the hormonal programs used, nor did any effect of age appear on the birth rates and survival rates in the study groups, including the control group, where all three aforementioned

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parameters recorded 100% response rates to the study protocols (Figures 4,5,6).

Table 1. relationship between age of tested ewes and multiple births in different groups.

Groups	Age interval	Total No.	Type of birth			X2/P value
			single	twin	Triple	
Control	Less than 4	4	2(100)	0(0)	0(0)	0/1
	≥ 4 years	6	4(66.6)	0(0)	0(0)	
G1	Less than 4	1	-	-	-	-
	≥4 years	9	4(44.4)	5(55.5)	0(0)	
G2	Less than 4	4	3(75)	1(25)	0(0)	1.21/ 0.27
	≥4 years	6	1(16.6)	2(33.3)	0(0)	
G3	Less than 4	1	1(100)	0(0)	0(0)	0.32/ 0.57
	≥4 years	9	5(55.5)	2(22.2)	0(0)	
G4	Less than 4	7	4(57.1)	2(28.5)	0(0)	0.178/ 0.67
	≥4 years	3	1(33.3)	1(33.3)	0(0)	
G5	Less than 4	4	1(25)	0(0)	1(25)	3.32/ 0.19
	≥4 years	6	3(50)	2(33.3)	0(0)	
G6	Less than 4	6	2(33.3)	1(16.6	0(0)	0.13/ 0.70
	≥4 years	4	1(25)	1(25)	0(0)	

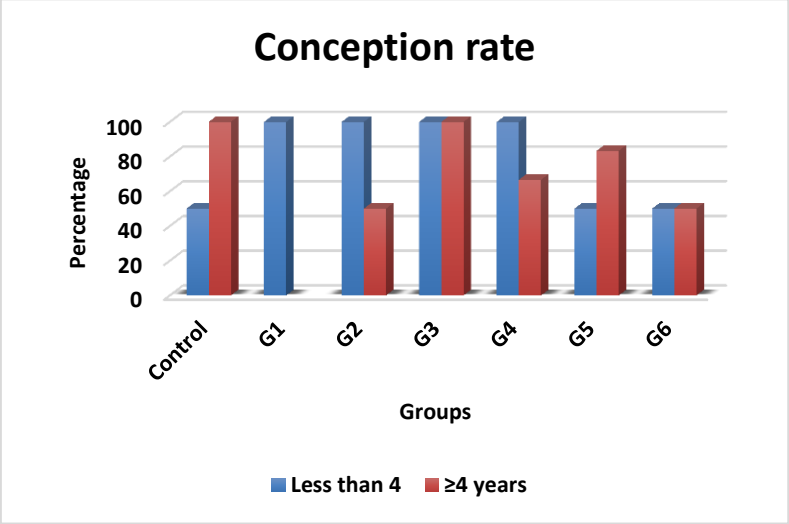

Figure1. Relationship between age of tested ewes and conception rate in different groups.

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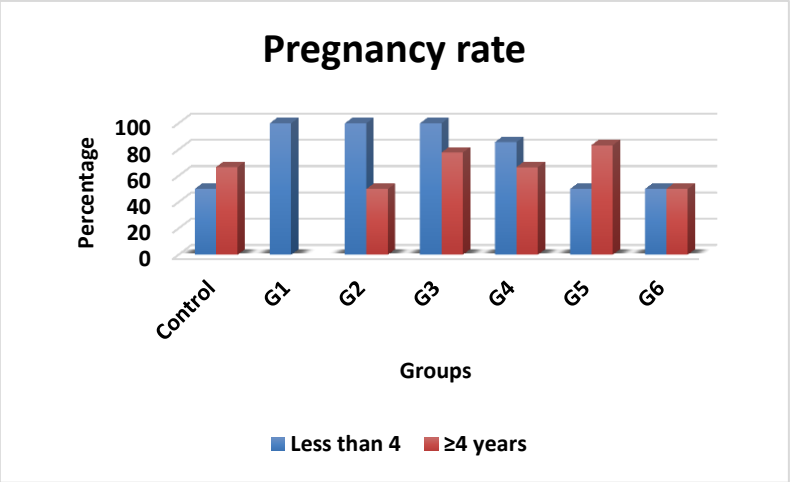

Figure2. Relationship between age of tested ewes and pregnancy rate in different groups.

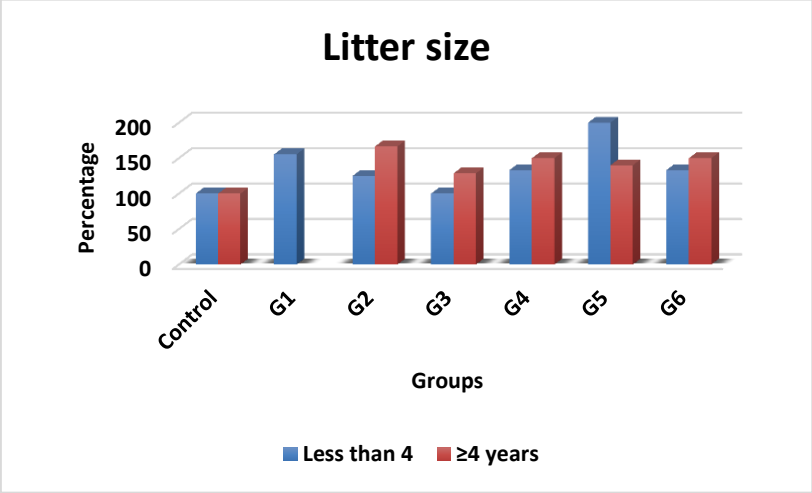

Figure3. Relationship between age of tested ewes and litter size rate in different groups.

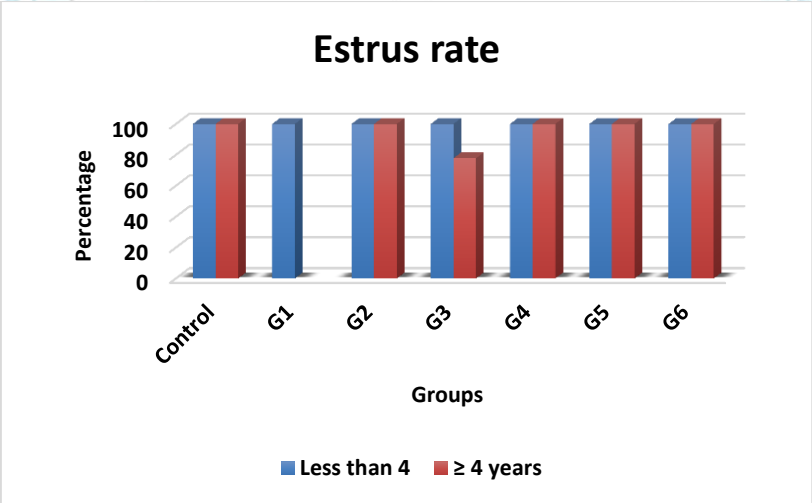

Figure 4.Estrus rate in relation to age of tested ewes

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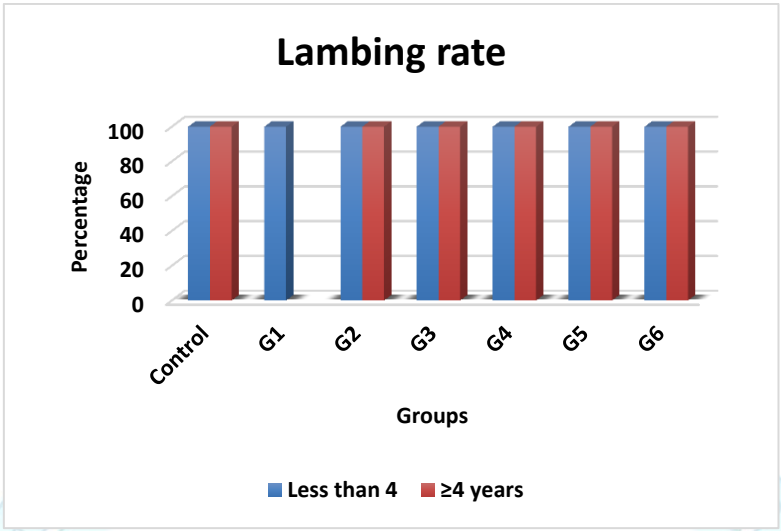

Figure 5.Lambing rate in relation to age of tested ewes

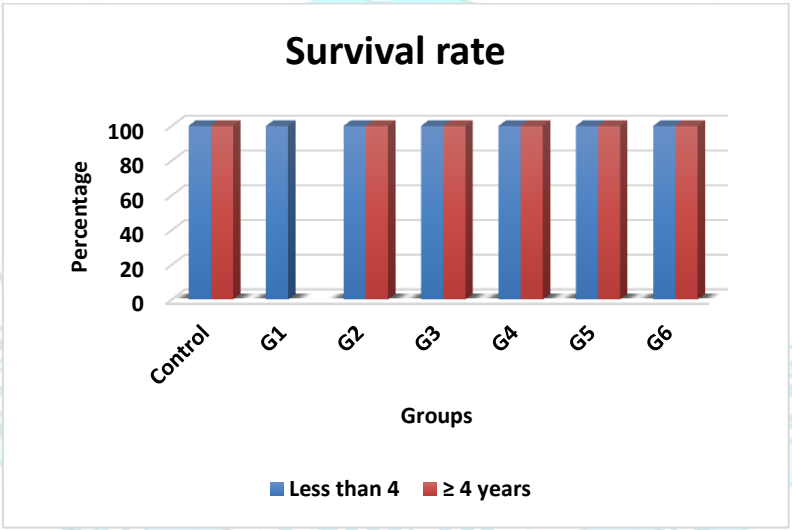

Figure 6.Survival rate in relation to age of tested ewes

Discussion

Age and many other factors had an impact on the reproductive activity, which in turn affected the breeding of huge numbers of ewes (9). In this study, ewes aged 4 years and over showed superior rates of twin births compared to those aged less than 4 years, although the difference was not significant between the two age categories (Table1). The reason for this superiority may be due to the fact that most of the ages of the ewes tested in all study groups are 4 years old, and this age is the best at which ewes reach sexual maturity (the age at which ewes are at the peak of their reproductive production) as a result of the optimal activity of their endocrine system. This result is somewhat similar to the result of (22) in the same Iraqi Awassi ewes, where they indicated that the best

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age for ewes used in superovulation programs to produce twins in this breed is limited to (2-4) years, noting that the aforementioned researchers used the long-term vaginal progesterone sponge protocol only, which is the same protocol that recorded the highest twinning rate (55.5%) in the current study. However, the result of this study contradicts (23), which did not record any differences between standardized groups in Pirlak sheep that aged between 2-5 years, and and (24) in Rmanov ewes aged 2-3 years. This is due to the fact that the study of these researchers was not designed to explore a relationship between the age of the ewes used and various fertility parameters.

Most of the groups subject to the study protocols recorded superiority for those aged less than four years in the parameters of conception rate and pregnancy rate, with the opposite in respect to litter size rate, when all groups recorded superiority for age category of 4 years and older (except for G1 and G5). The reason for the superiority of ewes aged 4 years and over in the litter size parameter may be due to the integration of the activity of the endocrine system and the reproductive system of these ewes that have reached the age of sexual maturity compared to the age groups under 4 years. The other probable reason for these differences is the differences in responses according to protocol applied. In general, the good response to different fertility parameters in different groups and different age categories may be due to the fact reported by (25) in that the eCG can increase pregnancy and twinning rates in breeds characterized by low litter size. This fact is applies perfectly on Awassi ewes which naturally have twin ratio of 5% only (26).

Conclusions

The current study was conclude that there are good indicators for the effect of the age of ewes subjected to superovulation protocols on most fertility parameters, and that the best age that gave the best response to the most important parameters, especially the rate of twinning and the litter size, is the age of 4 years. Therefore, we recommend selecting ewes of ages limited to 3-4 years for superovulation protocols.

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Declaration of Conflict of Interest

the authors declare that There is no conflict of interest

Ethical of approval

This research complies with the ethics rules of the University of Al-Qadisiyah, Iraq's Faculty of Veterinary Medicine ethics permission number: 4/9/2023).

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