

Research Article

Age as a Determinant of the Effectiveness of Intravaginal Sponges Impregnated with Progestin and Equine Chorionic Gonadotropin for Multiple Births Induction of Awassi Ewes

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The present study was conducted to evaluate the role of age in the effectiveness of intravaginal progestin-impregnated sponges (IPIS) along with equine chorionic gonadotropin (eCG) on the multiple birth rates of Awassi ewes. One hundred mature ewes (live weight was 35–40 kg and age 2–5 years) were allocated to three groups according to their ages ($n = 29$, 2–3 years; $n = 34$, 3–4 years; and $n = 37$, 4–5 years). All ewes were impregnated with IPIS, injected with 20 mg cronolone, for 14 days, and eCG (400 IU, i.m.) at sponges withdrawal. All treated ewes came to estrus (100%); the highest twinning rate was recorded in ewes aged 2–3 years, while the highest triple rate were found in those aged 3–4 years. A negative correlation coefficient was recorded between the age of treated ewes and the multiple births. In conclusion, the best age of Awassi ewes for estrus synchronization programs and multiple birth outcomes using IPIS along with eCG is 2–4 years.

1. Introduction

The bulk of domestic sheep in central climate zones are seasonal polyestrous animals [1]. The breeding season for sheep displays regional differences [2]. In ewes, the maximum level of follicle stimulating hormone (FSH) does not differ significantly between in-breeding and out-breeding seasons; however, the maximum level of luteinizing hormone (LH) is much greater in the in-breeding season than in the out-breeding season [3]. Several approaches of estrus synchronizing of ewes have already been tested, with varying levels of success [4–6]. In most cases, intravaginal sponges impregnated with medroxyprogesterone acetate (MAP) or fluorogestone acetates (FGA) are used to synchronize estrus in sheep [3]. Aside from the fact that approximately 90% of sheep exhibit estrus, only around half of them conceive and deliver as a consequence of breeding after induced estrus

using such methods [5, 7, 8]. The standard treatment procedure for intravaginal progesterone in ewes is a 14-day therapy protocol even if melatonin is being used to promote the mating outside the breeding season, it is critical to administer eCG at the device removal, but that is not the case during the breeding season, despite the reality that eCG will help in synchronization [9]. Utilizing intravaginal progestin polyurethane sponge or progestin silicon-based device (CIDR), long-term (12–14 days) or short-term (6–7 days) protocols are utilized to promote and synchronize estrus and ovulation in ewes [10]. To promote estrus synchronization, follicular development, ovulation rate, and fertility, eCG is given in doses ranging from 300 to 600 IU, depending on the animal's bodyweight, breed, and time of the year [4, 7, 11]. Ewes can breed at a faster rate with proper reproductive control, which would be more sustainable with farm output [12].

Progestin-impregnated vaginal sponges have been frequently used for estrus synchronization of ewes during breeding and nonbreeding seasons [2]. GnRH is utilized in estrus synchronization protocols to schedule ovulation with artificial insemination [13].

There is no previous studies focused on the efficacy of the multiple births in relation to age of tested synchronized ewes. This study aims to investigate the efficacy of using long-term protocol of progesterone with eCG at the time of withdraw of progesterone to produce multiple births within the breeding season and study the correlation between the age of synchronized ewes and multiple births to determine the best age for estrus synchronization programs.

2. Materials and Methods

2.1. Animals. One hundred healthy and cyclic Awassi ewes (weighed 35–40 kg, aged 2–5 years) raised by owners in Thi-Qar province, in Iraq, were used in this study. All the experimental animals were grazing freely in the pasture, in addition to the crushed barely diet in the evening.

2.2. Experimental Design. One hundred ewes were divided into three groups according to their ages as follows: 1st group—the 29 ewes aged (2-3) years, 2nd group—34 ewes aged (3-4) years, and the 3rd group—37 ewes aged (4-5) years. All ewes were treated with IPIS for 14 days with eCG injected at the time of sponges removal. The estrus response detected by standing to ram to be mated, conception response, type of births, abortion, and failure to conceive by returned to estrus were studied in ewes of all treated groups of the study. The results were compared with a control group including 35 untreated ewes at the same weight and ages of the treated ewes selected from the same flock which all mated naturally at the same way of the treated animals.

2.3. Hormonal Treatments. The ewes were treated with intravaginal sponges (20 mg cronolone, Intervet, Netherlands) for 14 days plus 400 IU of equine chorionic gonadotropin (Chronogest® eCG 6000 IU, Intervet, Netherlands) administered i.m at sponge withdrawal [9].

2.4. Mating. Ten fertile Awassi rams (one ram/10 ewes) weighed 50 kg were introduced to all ewes at twice with 6 hours intervals, starting about 20 hours after sponge withdrawal, and left with them for natural mating. Ewes were continuously monitored when rams were exposed to them for ensuring mating. The estrus response rate (ERR), conception rate (CR), lambing rate (LR), and twinning rate (TR) were calculated according to [14] by following equations:

$$\begin{aligned} \text{ERR} &= \left(\frac{\text{number of ewes expressing estrus}}{\text{total number of ewes}} \right) \times 100, \\ \text{CR} &= \left(\frac{\text{number of ewes that conceived}}{\text{number of inseminated ewes}} \right) \times 100, \\ \text{LR} &= \left(\frac{\text{number of lambs born alive}}{\text{number of ewes that conceived}} \right) \times 100, \\ \text{TR} &= \left(\frac{\text{number of ewes having twins}}{\text{number of ewes giving birth}} \right) \times 100. \end{aligned} \quad (1)$$

2.5. Statistical Analysis. Statistical analysis for data was done using Statistical Package of Social Sciences (SPSS), version 27, (Inc., Chicago, IL, USA) computer software. Statistical differences among groups and age intervals were analyzed and performed using the chi-square test, $P < 0.05$ was regarded as statistically significant [15].

3. Results

The effect of estrus synchronization protocol with progestin sponges with eCG on estrus response, type of births (singular, twins, and triples), and conception rate were studied. The estrus response rate was 100%, with a conception rate of 95% (92% successful pregnancy until lambing and 3% aborted), while the rest 5% failed to conceive (Table 1). The results revealed a significant ($P < 0.05$) superiority in twin births (77.17%) on triple and singular births (Table 2).

The total number of lambs born in all treated ewes in this study was 178 lambs with the highest ratio of twins in ewes aged from 2-3 years and the highest triplets lambs in those aged from 3-4 years namely 89.66 and 17.65, respectively (Table 3). On the other hand, there was a negative relationship between the age of treated ewes and the type of birth, i.e, there was a decrease in the multiple births with the advancement of the age (Figure 1).

4. Discussion

Intravaginal sponges containing progestin are among the most commonly used therapies for estrus synchronization in small ruminants [1]. The most widely used techniques include using intravaginal progestagen-releasing pessaries and then administering eCG or PMSG after the pessaries are removed [16]. In the current study, we tried to find out the suitable age of Awassi ewes which respond to IPIS along with eCG administration to increase the ovulation rates, as we know that Iraqi Awassi ewes are associated with low fecundity [17, 18] as the ewes always fail to conceive during the breeding season. Previous study on Iraqi Awassi ewes [19] showed the possibility of fecundity enhancement.

TABLE 1: Pregnancy, abortion, and pregnancy failure rates of Awassi ewes impregnated with intravaginal progesterin sponges along with eCG and control group.

Groups	Pregnancy rate		Abortion rate		Conception failure rate	
	No	%	No	%	No	%
Treated ewes (<i>n</i> = 100)	92	92	3	3	5	5
Control (<i>n</i> = 35)	35	100	0.0	0.0	0.0	0.0
<i>X</i> ²				2.97		
<i>P</i> value				0.226*		

*Significant difference at *P* < 0.05.

TABLE 2: The numbers and percentages of singular, twins, and triplets lambs born of Awassi ewes impregnated with intravaginal progesterin sponges along with eCG treatment.

Total number of successful pregnant ewes	Singular		Twins		Triplets	
	No	%	No	%	No	%
92	9	9.78a	71	77.17b	12	13.04a
<i>X</i> ²				119.57		
<i>P</i> value				0*		

*Significant difference at *P* < 0.05.

TABLE 3: The percentages of lambs born according to the type of birth related to the age of Awassi ewes impregnated with intravaginal progesterin sponges along with eCG and the control group.

Age (year)	Groups							<i>x</i> ²	<i>P</i>
	Control			Treated					
	Single	Twin	Triplet	Single	Twin	Triplet			
2-3	18 (100%)	0.0 (0.0%)	0.0 (0.0%)	1 (3.45)	26 (89.66)	2 (6.89)	42.99	0.0	
3-4	10 (100%)	0.0 (0.0%)	0.0 (0.0%)	0.0 (0.0)	28 (82.35)	6 (17.65)	44	0.0	
4-5	7 (100%)	0.0 (0.0%)	0.0 (0.0%)	8 (27.59)	17 (58.62)	4 (13.79)	12.16	0.002	
<i>x</i> ²		—			17.31				
<i>P</i>		—			0.0				

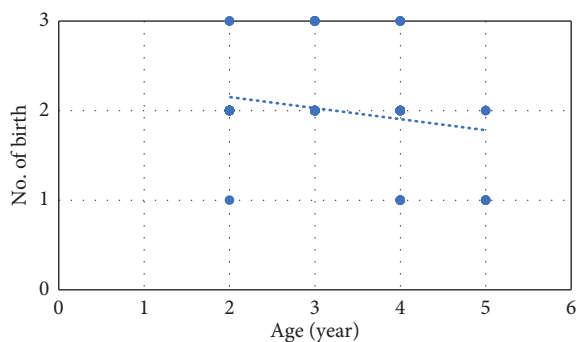


FIGURE 1: Relationship between age of Awassi ewes and type of birth of ewes impregnated with intravaginal progesterin sponges along with eCG. *R* = -0.224 (no significant difference).

The role of progesterone in the sponge is to inhibit GnRH and LH release via negative feedback, and once the progesterone level declines rapidly, after sponge withdrawal, the LH wave level rises considerably, and the dominant follicles ovulate [20, 21]. Due to these activities in sheep, progesterone has been used in estrus synchronization techniques [3].

The current results demonstrated a high conception rate (95%) after estrus synchronization with progesterin-impregnated sponges and eCG injection. This result is agreed with those reported by Amer and Hazzaa [22], who found the pregnancy rate about 91.7% and were also consistent with the results of Zarkawi [23], who use a similar protocol on Syrian Awassi ewes and recorded 100% conception rate, and Abdulkareem et al. [6], who obtained 100% conception rates for Awassi ewes in 9 Iraq provinces. On the other hand, the current results were greater than the conception rate recorded by Alkass et al. [7], 84.3% for Awassi ewes (*n* = 293). Moreover, the authors obtained A 130.7 lambing rate and A 26.8% twinning rate for similar ewes.

But Zarkawi [23] recorded a low twinning rate (50%) in comparison to our results (77.17%); however, he suggested that utilizing IPIS for estrus synchronization in Syrian Awassi ewes throughout the breeding season would be useful, particularly the idea of using eCG to increase the twinning rate, while Abdulkareem et al. [6] recorded a higher twinning rate (88–89.5%) for Awassi ewes in Nineveh and Anbar Iraqi provinces.

The current results confirmed the efficiency of the present protocol in improving and expanding the sheep

population because the ratio of twins is only 5% among Awassi ewes [24], while some researchers also suggested the efficacy of long-term progesterone protocol in both estrus synchronization and pregnancy rates [8, 12, 25], while other recorded a low rate of pregnancy (40%) only when a similar protocol of synchronization on Pirlak ewes was used out of the breeding season [3]. The present multiple births were higher than that recorded by other researchers [1, 2] who recorded a pregnancy rate of 86.7% and 75.6% in Akkaraman and Kivircik ewes, respectively, and 95–212% in Iraq Awassi ewes in 12 provinces [6]. These differences may be attributed to the differences in the breed of ewes tested, or to the variation of the differences in type and potency of progestins and/or eCG used.

Our results, on the other hand, showed a relationship between the age of treated ewes at twinning rate since better results were revealed in the ewes aged 2–4 years. Accordingly, the present protocol can be recommended to be applied in the ewes aged 2–4 years for estrus synchronization and induction of multiple births.

5. Conclusions

This study revealed that long-term IPIS combined with eCG injection at the time of sponge withdrawal is an efficient program for the production of multiple births in Awassi ewes, and that the ideal age to utilize in such synchronization programs is between 2 and 4 years.

Data Availability

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Ethical Approval

The current work has never been published in any language, nor is it being considered for publication in a similar or identical form by any other peer-reviewed journal. The animal experiments were conducted with approval from the College of Veterinary Medicine's Ethical Committee (Ref. No. 23/2022). Prof. Dr. Qassem Halim Kashash (Chairman), Asst. Prof. Dr. SAmir Ahmed Abdel Reda (Vice-Chairman), Asst. Prof. Dr. Saad Hashem Ghamis (member), Asst. Prof. Dr. Wissam Hussein Salman (member), and Abbas Fadhil Daham constitute the Ethical Committee (members).

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Authors' Contributions

Abbas Fadhil Daham and Ali Habeeb Jaber were concerned with conceptualization and methodology; Abbas Fadhil Daham, Ali Habeeb Jaber, and Jabbar Abbas Al-Saaidi conducted formal analysis; Abbas Fadhil Daham, Ali Habeeb Jaber, and Jabbar Abbas Al-Saaidi were responsible

for investigation and data curation; Ali Habeeb Jaber and Jabbar Abbas Al-Saaidi contributed to the study validation; Abbas Fadhil Daham and Jabbar Abbas Al-Saaidi were involved in the visualization and original draft preparation; Abbas Fadhil Daham, Ali Habeeb Jaber, and Jabbar Abbas Al-Saaidi worked on writing review and editing; Abbas Fadhil Daham assumed supervisory responsibilities; Fadhil Daham and Ali Habeeb Jaber followed project administration; Abbas Fadhil Daham was responsible for funding acquisition. All authors gave approval to the final version of the manuscript.

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