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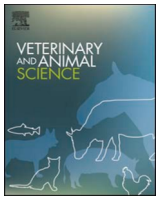

Evaluating estrus synchronization and early pregnancy detection in Ossimi sheep: The influence of fluorogestone acetate treatment duration and dosage

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ABSTRACT

Estrus synchronization is important for improving sheep reproduction. To enhance sheep reproduction efficiency, this study investigated the impact of different durations (7 vs. 14 days) and fluorogestone acetate (FGA) doses in intravaginal sponges on estrus synchronization and early pregnancy detection in Ossimi sheep. Two hundred ewes were evenly divided into two groups, each receiving a full 40 mg or a halved 20 mg FGA sponge for their respective durations. The study aimed to optimize breeding efficiency by examining the effectiveness of these treatments in synchronizing estrous cycles and by evaluating the use of serum levels of pregnancy-associated glycoprotein 1 (PAG1) and progesterone (P_4) as markers for early pregnancy identification. Prostaglandin $F_{2\alpha}$ and equine chorionic gonadotropin were administered to enhance the synchronization process. Results highlighted that the 7-day treatment protocol significantly improved estrus, pregnancy, and lambing rates compared to the 14-day protocol. Furthermore, pregnant ewes demonstrated elevated levels of PAG1 and P_4, with PAG1 levels particularly higher in ewes with multiple pregnancies. The findings underscore that the shorter duration of FGA treatment is more effective for reproductive management in Ossimi sheep without significantly affecting PAG1 levels based on the dose or duration of FGA. PAG1 also proved to be a reliable marker for early pregnancy detection, offering a promising approach to identifying fetal numbers early in pregnancy. This research suggests optimizing FGA sponge use could be cost-efficient for improving reproductive efficiency and early pregnancy management in sheep.

1. Introduction

Estrus synchronization is a crucial reproductive strategy to enhance sheep reproduction (Garoussi et al., 2020). The most commonly used method for synchronizing sheep's estrus is an intravaginal sponge impregnated with progestogens (Abecia et al., 2012). The majority of recent research has focused on the duration of treatments (Silva et al., 2020); over the past two decades, significant research has focused on the

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application of progesterone in sheep production, particularly regarding its impact on reproductive efficiency, estrus synchronization, and lambing outcomes. While studies have demonstrated the hormone's efficacy in improving reproductive metrics (Bartlewski et al., 2017), gaps remain in our understanding of the long-term effects on ewe health and productivity, as well as the need for breed-specific protocols to optimize outcomes (Bairagi et al., 2018). Furthermore, research has yet to fully address the welfare implications of progesterone delivery methods and their interaction with environmental and management factors (Gonzalez-Bulnes et al., 2020). Addressing these gaps requires a multidisciplinary approach, incorporating insights from veterinary medicine, animal science, and welfare studies, to develop more refined and welfare-friendly reproductive management strategies in sheep production. Which can be classified as short-term or long-term procedures and last 6 to 14 days. Even though manufacturers still choose traditional long-term protocols far more (Gonzalez-Bulnes et al., 2020), short-term procedures are currently widely utilized for the artificial insemination of sheep in the field. In both breeding and non-breeding seasons, short-term procedures are as successful as long-term ones at causing viable estrus and ovulation (Eldomany et al., 2023). Equine chorionic gonadotropin (eCG) has been shown to enhance reproductive performance by increasing the likelihood of pregnancy and superovulation while minimizing variations in estrus appearance following progesterone removal (Ghasemi-Panahi et al., 2016). Optimal fertility after synchronization may be achieved with lower progestagen levels by reducing the intravaginal sponge dose, according to specific theories (Algan et al., 2017; Di Giorgio et al., 2022), which reported that using intravaginal sponges impregnated with 15 mg of fluorogestone acetate (FGA) might result in more affordable estrus synchronization. Furthermore, this finding backs up another study (Di Giorgio et al., 2022), which claimed that a low dose of 20 mg of the FGA device is a simple and effective synchronization program for raising sheep reproductive capacity.

In the dairy industry, early pregnancy identification is a crucial tool in reproductive control, while predicting the number of fetuses enables the ewes in late pregnancy to get the proper nutrition, which reduces pregnancy toxaemia and the incidence of dystocia (Gearhart et al., 1988). During the gestation period, the mononucleate and binucleate cells of the ruminant trophectoderm generate pregnancy-associated glycoproteins (PAGs), which originate from the placenta (Sousa et al., 2006). Around day 18 or day 20 following breading, PAGs can be observed in the serum of pregnant ewes (Barbato et al., 2009) and are a reliable indication of pregnancy among ruminants, such as goats (Singh et al., 2021), sheep (Akköse et al., 2021), and cattle (Silva et al., 2007). Progesterone (P_4) is a crucial hormone for determining pregnancy in ewes (Schneider & Hallford, 1996). Numerous studies showed that multiple pregnancies often resulted in higher progesterone and PAG levels than single pregnancies (Barbato et al., 2018); moreover, PAGs and progesterone have a strong positive correlation (Roberts et al., 2017). Therefore, this study first examines the effects of reducing doses of FGA-impregnated intravaginal sponges during long- and short-term synchronization on the reproductive capacity of Ossimi ewes during the non-breeding season, as well as measures the serum levels of PAG1 and progesterone for early pregnancy assessment in ewes between 18 and 34 days.

2. Materials and methods

2.1. Ethical statement

The authors affirm that they have followed the journal's author standards and got the necessary ethical review committee approval following the journal's ethical norms. To ensure the safety of animals utilized in research, the authors attest that they have adhered to all applicable EU regulations and Kafrelsheikh University (KFS-IACUC/010/2022).

2.2. Animals and experimental design

Two hundred healthy Egyptian Ossimi ewes were selected with a good (2.5–3) body condition score (3–5 years old, 1–3 parities, and weighing 50–60 kg). The animals were randomly divided into two experimental groups with 100 ewes each. Group 1 was randomly divided into two subgroups (50 ewes each) and treated with a whole (40 mg FGA) sponge (Chronogest® Intervet International) for 7 and 14 days. Group 2 was randomly divided into two subgroups (50 ewes each) and treated with a halved sponge (20 mg FGA) for 7 and 14 days. All treated ewes in both groups received 250 μg of $PGF_{2\alpha}$ analogue (Cloprostenol sodium, Estrumate, Coopers Animal Health L.T.D., Berkhamsted, England) the day before sponge withdrawal and 600 IU of eCG (Folligon, Intervet, Germany) on the day of sponge withdrawal, as shown in Fig. 1. This research was conducted during the out-of-breeding season at the Riwina Animal Production Farm, Agricultural Research Center, Kafrelsheikh (Latitude 31° 06′ N, Longitude 30° 56′ E), Egypt (June-August 2022). The sheep were maintained in a semi-open yard, fed a concentrate feed combination and roughages following NRC (NRC, 2007) guidelines, and given unlimited access to fresh water.

2.3. Reproductive parameters

Following sponge removal, estrus detection was performed twice daily (every 12 h), and estrus symptoms were monitored using the vasectomized rams for five days. After confirmation of estrus, eight fertile rams (3–5 years old) were used to breed 152 ewes (1 ram/19 ewes). The effectiveness of the two protocols was assessed for estrus rate (No. of estrous ewes/No. of treated ewes × 100), the onset of estrus (h), pregnancy rate (No. of conceived ewes/No. of mated ewes × 100), lambing rate (No. of lambed ewes/No. of mated ewes × 100), and prolificacy (No. of lambs per lambed ewes).

2.4. Progesterone assay

Blood samples were collected from all ewes ($n = 200$) on days 0 and 6, and on day 13 only for long-term whole and halved sponge-treated ewes ($n = 100$) from the beginning of treatment before the breeding, and then on days 18, 22, 26, 30, and 34 after breeding, as shown in Fig. 1. Blood serum was kept at −20 °C until analysis. According to the manufacturer's instructions, the mini-VIDAS (VIDAS TESTS, BIOMÉRIEUX, France) was used for progesterone measurement. The range of the assay varied from 0.25 to 80 ng/ml, with an intra-assay variability of 4.12 % and an inter-assay variability of 6.32 %.

2.5. Pregnancy-associated glycoprotein one assay

Serum PAGs (200 samples) were collected every four days from days 18 to 34 after breeding, as shown in Fig. 1, and then quantified using a Sandwich ELISA Kit (Catalogue No. SG-70126; Sino Gene Clon Biotech Co., Ltd., China) following manual instructions for commercial kits. The cut-off value for PAG1 was 9.00 ng/ml, and PAG1 concentrations were calculated using a standard curve duplicated on each plate using a plate reader set at a wavelength of 650 nm. The conventional best-fit line is accepted if the fit R2 is more significant than 0.989. For the two quality control pools, the mean intra-assay CVs were between 3 and 5 %. The mean inter-assay CVs were 2 and 15 % for the same quality control pools. Ten ewes with similar age, parity, and genetic background were kept unmated to serve as controls. They were separated from the rams for a minimum of five months. PAG1 concentrations in these ewes were assessed at the same time intervals as in mated ewes, and they acted as non-pregnancy controls to evaluate the assay's specificity (Vandaele et al., 2005). A pregnancy diagnosis was confirmed by transrectal ultrasonography (Esaote, Europe B.V., Netherlands) 35 days after breeding to confirm the results of PAG1 and progesterone levels for an early pregnancy diagnosis.

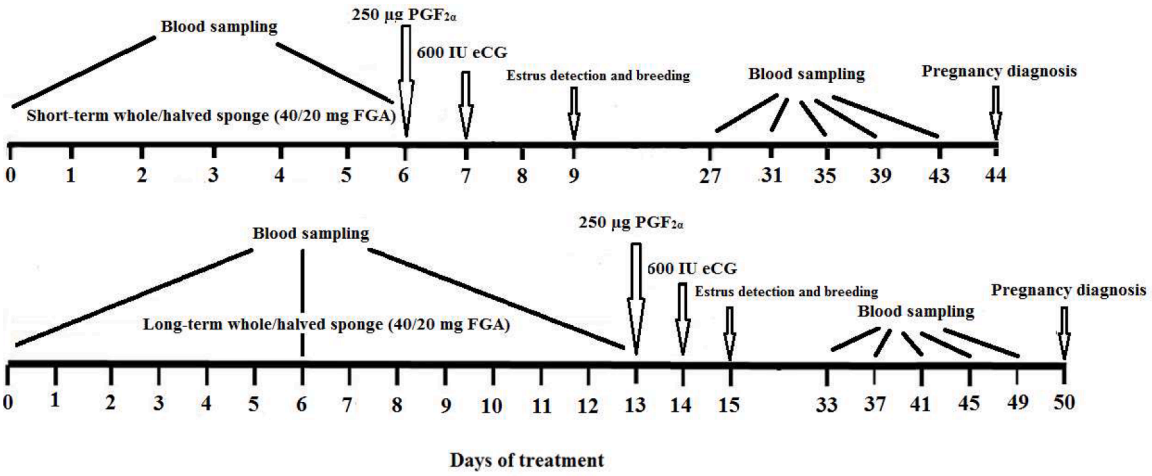

Fig. 1. Estrus synchronization experiment with whole and halved sponges. Ewes were synchronized using whole and halved sponges (40 and 20 mg FGA) for 7 and 14 days, with 250 µg of $PGF_{2\alpha}$ analogue (1 ml Estrumate) administered the day before sponge withdrawal and 600 IU of eCG (Folligon) administered on the day of sponge withdrawal. Blood sampling, estrus detection, breeding, and pregnancy diagnosis were conducted throughout this experiment.

2.6. Statistical analyses

Kolmogorov–Smirnov test was employed to check the data obtained for normality patterns. The obtained data were statistically assessed via a two-way analysis of variance for the onset of estrus and the 14-day treatment group and a three-way analysis of variance for P_4 measurements at days 0 and 6 treatments using SPSS (23) General Linear Model Procedure. In contrast, the Chi-square ($\chi 2$) test was used to analyze estrus, pregnancy, and lambing rates. Kruskal-Wallis was used to analyze prolificacy, followed by pairwise comparisons using the Steel-Dwass method. Statistical analyses of PAG1 and P_4 levels between and among sampling days were determined by repeated measures ANOVA. Differences between means were tested using Duncan's range multiple tests (Duncan, 1955).

3. Results

3.1. Estrus rate and onset of estrus

The short-term synchronization group had substantially higher estrus rates ($P < 0.05$) than the long-term sponge group. The highest estrus rate was found in ewes treated with a short-term halved sponge (96 %), followed by whole sponge (88 %), and the lowest in ewes treated with a long-term halved sponge (52 %; Table 1). The initiation of estrus showed notable variations among the most common synchronization procedures. The onset of estrus was the shortest ($P< 0.05$) in long-term sponge groups, whereas there were no significant differences between halved and whole sponge groups (Table 1).

3.2. Reproductive performance

Fortunately, there were neither abortions nor fetal deaths in this investigation; the lambing rate for each procedure was similar to the pregnancy rate (Table 1). According to the findings in Table 1, there were significant differences ($P < 0.05$) in pregnancy and lambing rates between short- and long-term sponge groups. In contrast, the short-term groups had higher pregnancy and lambing rates than the long-term groups. The halved sponge for 7 d has the most significant pregnancy and lambing rates (91.67 %), whereas the whole sponge for 14 d has the lowest rates (70.58 %). Higher prolificacy was recorded in the short-term (1.43) group compared to the long-term (1.32) group. The whole sponge for 7 d presented the highest prolificacy (1.44), followed by the halved sponge for 7 d (1.41) (Table 1).

Table 1
The reproductive efficiency of treated ewes during the non-breeding season.

	Item		*N*	Onset of estrus (h)	Estrus rate (%)	Pregnancy rate (%)	Lambing rate (%)	Prolificacy
Treatment	Halve sponge		100	44.64	74	84.30	84.30	1.36
	Whole sponge		100	40.43	78	76.20	76.20	1.39
	SE			3.15	0.39	0.78	0.78	0.02
	P-value			0.36	0.50	0.13	0.13	0.45
Time	7 d		100	53.86^{a}	92^{a}	86.75^{a}	86.75^{a}	1.43^{a}
	14 d		100	31.21^{b}	60^{b}	73.75^{b}	73.75^{b}	1.32^{b}
	SE			0.72	1.16	1.25	1.25	0.02
	P-value			0.001	0.001	0.034	0.034	0.01
Interaction	Halve sponge	7 d	50	57.14^{a}	96^{a}	91.67^{a}	91.67^{a}	1.41^{a}
		14 d	50	32.14^{c}	52^{b}	76.92^{b}	76.92^{b}	1.30^{b}
	Whole sponge	7 d	50	50.57^{b}	88^{a}	81.82^{ab}	81.82^{ab}	1.44^{a}
		14 d	50	30.29^{d}	68^{b}	70.58^{b}	70.58^{b}	1.33^{b}
	SE			0.680	3.31	1.48	1.48	0.02
	P-value			0.001	0.001	0.09	0.09	0.01

N = Number of ewes; *d* = Day.
^{a,b,c,d} Values with different superscript letters in the same column differ significantly ($P < 0.05$).
The chi-square ($\chi 2$) test was used to analyze estrus, pregnancy, and lambing rates, followed by pairwise comparisons with the Bonferroni correction. Kruskal-Wallis was used to analyze prolificacy, followed by pairwise comparisons using the Steel-Dwass method. Two-way analysis of variance for the onset of estrus.

3.3. Progesterone profile

No statistically significant differences were recorded in P_4 concentration on days 0 and 6 between the halved and whole sponge groups at 7 and 14 days for all treatment groups (Table 2). In contrast, all treated groups' P_4 concentrations were significantly higher on day 6 ($P < 0.05$) than on day 0. The P_4 levels in the long-term whole and halved sponge increased considerably ($P < 0.05$) on day 13 compared to the P_4 levels on days 0 and 6. Also, on day 13, no significant variations in P_4 concentrations existed between long-term whole and halved sponge groups (Table 2). Progesterone (P_4) concentrations were significantly higher in pregnant ewes compared to their non-pregnant counterparts ($P < 0.001$), with a notable trend where P_4 levels ascended from the lowest recorded values on day 18 to peak levels by day 34 of gestation (Table 3). A marked and highly significant interaction ($P < 0.001$) was observed between the state of pregnancy and the day of sample collection, demonstrating an increase in P_4 levels as the pregnancy advanced, applicable to single and multiple-pregnant ewes. Initially, on day 18, progesterone levels were found to be 3.91 ng/ml for single-pregnant and 4.04 ng/ml for multiple-pregnant ewes, which then rose to 4.81 ng/ml and 4.91 ng/ml, respectively, by day 34. In contrast, P_4 levels remained nearly unchanged in non-pregnant ewes during the same timeframe. Importantly, the increase in progesterone concentrations was more pronounced in ewes with multiple pregnancies than those with single pregnancies.

3.4. Serum PAG1 levels in pregnant and non-pregnant ewes

The PAG1 values were significantly higher ($P < 0.001$) in pregnant compared to non-pregnant ewes. Moreover, the progression in days of pregnancy greatly affected serum PAG1 concentrations, whereas the lowest value was recorded on day 18 and the highest on day 34 (Table 3). There is a highly significant ($P < 0.001$) interaction between pregnancy and sampling day, whereas PAG1 levels increased with the progress of the gestation period in both single and multiple-pregnant ewes (Table 3). The highest serum PAG1 concentration was recorded at day 34 in single and multiple-pregnant ewes (37.43 ng/ml and 48.00 ng/ml, respectively). Serum PAG1 levels were practically constant in non-pregnant ewes between days 18 and 34. Furthermore, serum PAG1 levels were significantly higher ($P < 0.05$) in multiple-pregnant than single-pregnant ewes from day 18 to day 34 after breeding during the whole sampling period (Table 3)

3.5. Effects of dose of FGA (whole-halved sponge) and treatment time (7–14 d) on PAG1 and P_4 concentrations and interactions

In Table 4, it is demonstrated that throughout the sampling period (18 to 34 days of gestation), the dose of FGA had no statistically significant effects on PAG1 and P_4. Long and short protocols (14–7 d) exhibit no significantly different effects on PAG1 during the sampling period, whereas P_4 concentration was markedly higher in the 7d protocol compared to the 14d protocol. According to the results, there is no significant interaction between the dose of FGA (whole-halved sponge) and treatment period (7–14 d) on the concentrations of PAG1 and P_4 during the sampling period (Table 4).

Table 2
The P_4 (ng/ml) concentration (mean ± SEM) of experimental ewes.

Treatment	N	0 d	6 d	13 d
Halve sponge 7 d*	50	0.82 ± 0.04^{b}	1.96 ± 0.11^{a}	
Halve sponge 14 d*	50	0.87 ± 0.03^{c}	1.91 ± 0.05^{b}	2.30 ± 0.08^{a}
Whole sponge 7 d*	50	0.84 ± 0.03^{b}	1.81 ± 0.03^{a}	
Whole sponge 14 d*	50	0.78 ± 0.05^{c}	1.87 ± 0.11^{b}	2.21 ± 0.09^{a}

N = Number of ewes; d = Day.
^{a,b,c} Values with different superscript letters in the same row differ significantly ($P < 0.05$). Values with the same * in the column do not differ significantly ($P < 0.05$). A three-way analysis of variance for P_4 measurements at days 0 and 6 treatments using SPSS General Linear Model Procedure.

4. Discussion

Estrus synchronization is essential for successful reproductive management, especially for conservation through population growth (Gibbons et al., 2019). During both the breeding and non-breeding seasons, it has been observed that short-term techniques are effective at inducing and synchronizing estrus (Eldomany et al., 2023). This research showed that using short-term protocols with whole and halved sponges achieved a higher estrus response rate (88 and 96 %, respectively) in comparison to long-term synchronization (68 and 52 %, respectively), in line with (Malik et al., 2021). In contrast, estrus began sooner in long-term synchronization than in short-term synchronization. This could be because the onset of estrus in a short-term protocol is often delayed since there is a developing follicle, so it needs some time to stimulate ovulation following progesterone removal (Oliveira et al., 2016). In contrast, in a long-term protocol, progesterone levels quickly decline once the device is withdrawn, and ewes initiate estrus and ovulate more rapidly (Nakafeero et al., 2020). Short-term synchronization is preferable to long-term synchronization in terms of pregnancy, lambing, and prolificacy (Menchaca et al., 2017). This could be because prolonged progesterone therapy interferes with oocyte development by raising sub-luteal progesterone levels and increasing the frequency of LH pulses without causing an LH surge, which inhibits the biggest follicle from ovulating (Özyurtlu et al., 2011) and causes older oocytes to ovulate (Viñoles et al., 2001). On the other hand, short-term progestagen protocols are linked to a decreased incidence of vaginitis, whereas employing intravaginal sponges for long periods was also linked to the presence of purulent and fetid vaginal discharges upon their removal (Vasconcelos et al., 2016), Long-term use of intravaginal sponges for sheep estrus synchronization, particularly for 12–14 days, is linked to negative outcomes such as vaginitis and purulent discharge. A study in Animals found that almost all treated ewes had vaginal discharge upon removal, significantly more so after 14 days, potentially reducing fertility by altering vaginal microbiota. The CIDR device, designed for better drainage, may offer a safer alternative by reducing infection and inflammation risks (Martinez-Ros et al., 2018). Moreover, the employment of progestogen in the devices appears to have a beneficial effect by increasing the alpha-diversity of the vaginal microbiota and decreasing the abundance of harmful microorganisms, suggesting a positive impact on fertility (Reinoso-Peláez et al., 2023) which affects the fertilization process. According to the manufacturer, the minimal dose that produces a high pregnancy rate and high prolificacy of ewes is 20 mg of FGA (Di Giorgio et al., 2022). Di Giorgio et al. (2022) revealed that the 20 mg FGA device (a halved sponge) is a simple and effective synchronization treatment to raise sheep fertility. This may be because the animal did not fully absorb the progestagen dose employed in sponges during the treatment period; nearly 1/4 of the progestagen from the whole sponge and 1/2 of the progestagen from the half sponges were absorbed (Greyling et al., 1997). In addition, decreasing the dose of FGA from 40 to 20 mg was adequate for promoting follicular dynamics, ovulation, and the growth of functional corpora lutea (Letelier et al., 2009). In line with other studies, it was reported that, compared to the whole sponge group, the halved sponge group exhibited greater conception and lambing rates (Algan et al., 2017).

This study reported that there was a significant increase in progesterone levels in both experimental groups following the start of the protocol ($P < 0.05$); this may be attributable to the insertion of the sponge, which caused progesterone to be released slowly over a prolonged period while remaining in the vagina (Kusina et al., 2000). We found that at 13 days after insertion for the long-term protocol, progesterone concentrations reach their maximum, which can negatively affect pregnancy due to high progesterone concentrations (Kaşikçi et al.,

Table 3
Mean and standard error of PAG1 and P_4 among different pregnancy statuses at different times.

Pregnancy status		N	18 d	22 d	26 d	30 d	34 d
Non-pregnant*	PAG1	76	5.14 ± 0.26^{a}	4.86 ± 0.26^{a}	5.14 ± 0.40^{a}	4.57 ± 0.37^{a}	4.71 ± 0.29^{a}
Single pregnancy**		76	18.71 ± 0.57^{e}	22.86 ± 0.86^{d}	26.14 ± 0.70^{c}	34.00 ± 0.62^{b}	37.43 ± 0.37^{a}
Multiple pregnancy***		48	23.29 ± 0.75^{e}	28.14 ± 0.74^{d}	32.86 ± 0.83^{c}	43.43 ± 0.75^{b}	48.00 ± 0.62^{a}
Non- pregnant*	P_4	76	0.91 ± 0.02^{a}	0.88 ± 0.02^{a}	0.90 ± 0.02^{a}	0.84 ± 0.03^{a}	0.85 ± 0.03^{a}
Single pregnancy**		76	3.91 ± 0.12^{d}	4.17 ± 0.08^{cd}	4.37 ± 0.05^{bc}	4.61 ± 0.07^{b}	4.81 ± 0.07^{a}
Multiple pregnancy**		48	4.04 ± 0.08^{d}	4.21 ± 0.03^{cd}	4.46 ± 0.06^{bc}	4.70 ± 0.07^{b}	4.91 ± 0.08^{a}

N = Number of ewes; d = Day; PAG1 = Pregnancy-associated glycoprotein 1 (ng/ml); P_4 = Progesterone (ng/ml).
Time groups within the same rows with different superscript letters show significant differences ($P < 0.05$). ^{a,b,c,d,e} Pregnancy status with different asterisk numbers show significant differences ($P < 0.05$). Statistical analyses of PAG1 and P_4 levels between and among sampling days were determined by repeated measures ANOVA. Differences between means were tested using the Range Multiple Tests of Duncan (1955).

Table 4
Effects of dose of FGA (whole-halved sponge) and treatment time (7–14 d) on PAG1 and P_4 concentrations and interactions between them in treated ewes.

Item		N	P_4	PAG1
Treatment	Halve sponge	64	4.26	30.67
	Whole sponge	60	4.28	30.79
	SEM		0.04	1.16
	P-value		0.68	0.94
Time	7	80	4.38^{a}	31.53
	14	44	4.16^{b}	29.94
	SEM		0.04	1.16
	P-value		0.001	0.335
Treatment × Time	Halve sponge 7 d	44	4.43	31.77
	Halve sponge 14 d	20	4.09	29.57
	Whole sponge 7 d	36	4.34	31.29
	Whole sponge 14 d	24	4.23	30.31
	SEM		0.06	1.64
	P-value		0.05	0.71

N = Number of ewes; d = Day, FGA = Fluorogestone acetate; PAG1 = Pregnancy-associated glycoprotein 1 (ng/ml); P_4 = Progesterone (ng/ml).

2011).

According to the present study, progesterone and PAG1 levels help identify pregnancies in sheep since they are in higher concentrations in pregnant ewes than in non-pregnant ewes. Our study reported that the PAG1 concentration had higher accuracy in early pregnancy diagnosis than the progesterone concentration, in line with Karen et al. (2003). This may be due to PAG1′s ability to distinguish between pregnancy and extended inter-estrus intervals. In contrast, progesterone measurements have low diagnostic accuracy for non-pregnant ewes (Susmel & Piasentier, 1992), which may be due to the early embryonic death or uterine and/or ovarian disease being the cause of the false positive instances (Karen et al., 2001). Although there is a positive interaction between pregnant ewe progesterone levels and days of pregnancy, progesterone levels were unable to differentiate between single and multiple early pregnancies, which is consistent with Kalkan et al. (1996), who reported a positive correlation between the number of fetuses and mean plasma progesterone concentrations only at the last half of pregnancy but not in early pregnancy, as well as the blood progesterone levels, which may categorize litter size in ewes at nearly the last two-thirds of the gestation period (Schneider & Hallford, 1996). Our findings showed that serum PAG1 levels were significantly higher ($P < 0.05$) in multiple-pregnant than single-pregnant ewes from day 18 till day 34 after breeding during the whole sampling period, which was consistent with other studies finding that PAG concentration was higher in multiple pregnancies in ewes than in single pregnancies (De Carolis et al., 2020). This may be due to the increased number of cotyledons with a higher surface area (Kaulfuss et al., 2000) and the subsequent secretory activity of twin placentas (Ranilla et al., 1997). Our research showed that in Ossimi ewes, serum PAG1 could distinguish between pregnant and non-pregnant individuals and single and multiple pregnancies by day 18 of pregnancy.

Moreover, this is consistent with other research findings showing that PAGs can distinguish between pregnant and non-pregnant ewes at day 18 (De Carolis et al., 2020). Another study showed that PAGs in the blood of different breeds of ewes were substantially identified at varying days of gestation, as early as 20 days (Karen et al., 2003) and 24 days (Barbato et al., 2009). This could be caused by variations in the breed and age of the ewes or in the pattern of ovine PAG concentrations, which vary according to the species (Barbato et al., 2018). Moreover, neither the dose of FGA nor the length (7–14) of the treatment periods had a statistically significant impact on the levels of PAG1 and P_4. PAG1 and P_4 levels increased considerably compared to non-pregnant ewes as the gestation period progressed. The study's limitations include the necessity for a more comprehensive examination of synchronization during the breeding season and a higher frequency of blood samples up to the 90th day of pregnancy for the detection of PAG1 and P_4 levels.

5. Conclusion

The findings of this study suggest that short-term estrus synchronization is more effective than long-term estrus synchronization in terms of estrus rate (%), pregnancy rate (%), lambing rate (%), and prolificacy. The halved sponge (20 mg FGA) is as effective as the whole sponge (40 mg FGA) in improving reproductive efficiency during estrus synchronization of Ossimi ewes. Serum PAG1 can distinguish between single and multiple pregnancies and is more accurate than progesterone in diagnosing early pregnancy in Ossimi ewes at 18 days of gestation.

Institutional review board statement

All experiments were performed according to the Kafrelsheikh University guidelines for the care and use of lab animals, and all of the steps meant to keep the animals from suffering were taken in code KFS-IACUC/150/2023.

Informed consent statement

Not applicable.

Data availability

Upon request.

Ethical statement

The authors affirm that they have followed the journal's author standards and got the necessary ethical review committee approval following the journal's ethical norms. To ensure the safety of animals utilized in research, the authors attest that they have adhered to all applicable EU regulations and Kafrelsheikh University (KFS-IACUC/010/2022).

CRediT authorship contribution statement

Maha S. Salama: Formal analysis, Data curation, Conceptualization. **Mohey A. Ashour:** Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Ehab S. Taher:** Conceptualization, Data curation, Investigation, Methodology. **Ismail El-kon:** . **Samy Sayed:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software. **Lamya Ahmed Alkeridis:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software. **Batrina Stefan:** Investigation, Methodology, Resources, Software, Validation. **Imbrea Ana-Maria:** Conceptualization, Resources, Validation, Visualization, Writing – review & editing. **Laila A. Al-Shuraym:** Funding acquisition, Investigation, Resources, Software, Writing – review & editing. **Mustafa Shukry:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software.

Declaration of competing interest

The authors have declared that there is no conflict of interest with respect to this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2024.100351.

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