DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia https://veterinariamexico.fmvz.unam.mx/

Comparison of estrus response and subsequent fertility following estrus synchronization with six protocols in Ossimi ewes during the early summer season

Essam A. Almadaly[1]*
0000-0003-3598-8090
Ferial Sahwan[2]
0000-0001-8176-4763
Wael B. El Domany[3]
Ahmed M. Fawzy[1]
Mustafa Shukry[4]
0000-0003-2722-2466
Foad Farrag[5]
0000-0002-4048-6717

[1] Kafrelsheikh University, Faculty of Veterinary Medicine, Department of Theriogenology, El-Geish Street, Kafrelsheikh 33516, Egypt.

[2] Alexandria University, Faculty of Veterinary Medicine, Animal Breeding and Production, Animal Husbandry and Wealth Development Department, Egypt.

[3] Mansoura University, Faculty of Veterinary Medicine, Department of Theriogenology, Mansoura, Egypt.

[4] Kafrelsheikh University, Faculty of Veterinary Medicine, Department of Physiology, El-Geish Street, Kafrelsheikh 33516, Egypt.

[5] Kafrelsheikh University, Faculty of Veterinary Medicine, Department of Anatomy and Embryology, El-Geish Street, Kafrelsheikh 33516, Egypt.

***Corresponding author:**
Email address:
dr_essam_2005@yahoo.com

Submitted: 2022-03-14
Accepted: 2022-10-27
Published: 2023-01-30

Additional information and declarations can be found on page 13

Abstract

This study investigates the effect of estrus synchronization on the reproductive performance of Ossimi ewes during the early summer season to prove that they are cyclic throughout the year in subtropical climates. In June, 280 ewes were assigned to seven (n = 40) groups and received one of the following: 1) short-term progesterone (ST) P4 treated ewes received 20 mg P4 day after day for 6 days and 500 international unit (IU) equine chorionic gonadotropin (eCG) on day 6; 2) long-term P4 (LT) P4 treated ewes received 20 mg P4 day after day for 12 days and 500 IU eCG on day 12; 3) double prostaglandin (PG) $F_{2\alpha}$ 7days apart and 500 IU eCG on day 7 ($PGF_{2\alpha}$-7d); 4) double $PGF_{2\alpha}$ 14 days apart and 500 IU eCG on day 14 ($PGF_{2\alpha}$-14d); 5) traditional ovulation synchronization (T-Ovsynch; gonadotropin-releasing hormone (GnRH)-0 day; $PGF_{2\alpha}$-5 day and GnRH-7 day); 6) non-traditional ovulation synchronization (NT-Ovsynch; GnRH-0 day; $PGF_{2\alpha}$-7 day and GnRH-9 day); 7) control ewes received no treatment. There were significant differences among groups for estrus rate, the onset of estrus, pregnancy, and lambing rates as well as prolificacy and gestation period. The highest (90 %) estrus rate was recorded in (LT) P4, $PGF_{2\alpha}$-14d, and NT-Ovsynch protocols while the lambing rate was the maximum (100 %) in $PGF_{2\alpha}$-14d. Although all treatments had a positive effect on ewe fertility; long-term protocols particularly $PGF_{2\alpha}$-14d; are recommended to improve Ossimi ewe fertility during the early summer season.

Keywords: Estrus synchronization; Ovsynch protocol; Ossimi ewes; $PGF_{2\alpha}$; Progesterone; Prolificacy.

Cite this as:
Almadaly EA, Sahwan F, Wael BE, Fawzy AM, Shukry M, Farrag F. Comparison of estrus response and subsequent fertility following estrus synchronization with six protocols in Ossimi ewes during the early summer season. Veterinaria México OA. 2023;10. doi: 10.22201/fmvz.24486760e.2023.1058.

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Study contribution

Sheep production is a capital investment in our country and Ossimi ewe fertility is reduced during the non-breeding season. The current study contributes to increasing sheep production during the transition period from the spring season to the summer season through the application of some hormonal treatments in an attempt to improve ewe fertility and produce lamb throughout the year.

Introduction

The majority of ewe breeds differ in their reproductive behavior depending on various factors such as season, the onset of puberty, the length of photoperiod, nutritional status or flushing, the ram effect, hormonal treatments as well as their follicular waves.[1] Ossimi breed is the most popular breed in Egypt where its population is over 1 000 000 head with higher productivity in middle Egypt than in Southern Egypt.[2] As a result, several strategies have been used to control the ovarian activity of small ruminants in trials to improve their *in vivo* fertility.[3] Low fecundity is the most common reproductive constraint in ewes, which causes great economic losses in the ovine industry.[4]

Reproductive scientists endeavor to achieve precise intervention of reproductive hormones with corpus luteum (CL) and follicular development to produce an optimized hormonal milieu in which all animals ovulated and conceived.[5] In small ruminants, hormonal estrus synchronization is achieved either by reducing the length of the luteal phase with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or by extending it artificially with either exogenous progesterone (P4) or more potent progestagens.[6]

The success to synchronize breeding and lambing in sheep farms as well as getting high fertility at the first service is highly profitable for sheep farmers.[7] Highly synchronized estrus and ovulation were achieved in ewes following a double $PGF_{2\alpha}$ protocol, 7 days apart.[8] However, this protocol yielded low reproductive outcomes in comparison with combined progestagen and equine chorionic gonadotropin (eCG) protocols.[9] This could be attributed to a variable hormonal milieu during the early luteal phase, which adversely affects the growth of follicles leading to lower ovulation, conception, and lambing rates.[10]

Fierro et al.,[11] proposed that extending the time between double $PGF_{2\alpha}$ doses may increase the time that follicles are required to ovulate with exposure to sufficient P4 during its development; maximizing the fertility of these $PGF_{2\alpha}$-based protocols. Previous trials in sheep,[12,14] reported that administration of double $PGF_{2\alpha}$ from 10 to 16 days apart yielded different estrus responses and duration of the luteal phase, which affect follicular growth. Although long-interval double $PGF_{2\alpha}$ protocols (14 or 16 days apart) yielded lower estrus synchrony, it has a better hormonal profile when compared to short-interval double $PGF_{2\alpha}$ protocols (10 or 12 days apart).[12]

Low ovulatory rates to the first gonadotropin-releasing hormone (GnRH), regression of the dominant follicle before $PGF_{2\alpha}$, as well as spontaneous/premature luteolysis after the first dose of GnRH and before $PGF_{2\alpha}$ may all be contributing factors to the low reproductive success of ovulation synchronization (Ovsynch) protocols.[15] The dose of GnRH may also be a factor, as we previously reported[16]

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

that the common dose of GnRH analogue (4 µg buserelin acetate) used for estrus synchronization in ewes failed to induce estrus in out-of-season Rahmani ewes.

In cyclic ewes, the follicular wave ends when the largest follicle either ovulates or becomes atretic, leading to the onset of the next follicular wave.[17] Administration of GnRH 6 days before $PGF_{2\alpha}$ increases the conception rate[18] and the number of synchronized cows and reduces variations in the onset of estrus.[19] Moreover; the duration of this synchronization method is shorter in comparison with either progestagen-based or double $PGF_{2\alpha}$-based protocols. Additionally, it is practical because it excludes the unpleasant odor of intravaginal devices and the surgical insertion of an implant.[20] Application of Ovsynch protocols outside the breeding season is aimed to increase circulating P4 concentration by inducing ovulation and/or luteinization of follicles. However, various studies differed in timing, dosage, and route of treatment with GnRH analogues.[21, 22]

Outside the breeding season, P4 and eCG are prerequisites for priming the brain and for follicle growth, respectively.[23] Thus, 7 days interval between GnRH and $PGF_{2\alpha}$ may be more advisable for eCG treatment particularly, if follicular exposure to eCG from wave onset is wanted. Moreover, it has been proven that either cyclic or acyclic ewe has 3–4 follicular waves per each estrous cycle.[17] Based on all these premises, our study aimed to investigate the relative efficacy of six [short-term P4 (ST) P4; long-term P4 (LT) P4; double $PGF_{2\alpha}$, 7 days apart ($PGF_{2\alpha}$, 7d); double $PGF_{2\alpha}$, 14 days apart ($PGF_{2\alpha}$, 14d); traditional-ovsynch (T-ovsynch) and non-traditional Ovsynch (NT-Ovsynch)] synchronization protocols on Ossimi ewe fertility during the early summer season to prove that Ossimi breed of ewes is cyclic throughout the year under subtropical climatic conditions. Moreover, the reproductive outcomes of these long- and short-term synchronization protocols were compared.

Materials and methods

1. Ethical statement

All experimental procedures complied with the guidelines established by the Ethics Committee on Animal Experimentation of Kafrelsheikh University, Faculty of Veterinary Medicine (25–9–19–KSU). All animal experiments were conducted according to the ARRIVE guidelines (https://arriveguidelines.org).

2. Animals

Two hundred and eighty healthy ewes of the Ossimi breed (3–5 years old, weighing 45–55 kg), showing estrus signs after exposure to rams during March were identified and isolated from rams until June. All selected ewes had a good (2.5–3) body condition score and previous parturition (1–3). The present experiments were conducted on a sheep flock kept at Riwina Animal Production Station, during the early summer season from June to July (long daylight) with average temperatures of 35.5 °C and 19 °C. This station is situated in Kafrelsheikh (Northern Egypt, Latitude 31° 06′ N, Longitude 30° 56′ E). All animals were kept in an open yard and provided with concentrate feed mixture (CFM) and roughages following the standard requirements of the National Research Council.[24] The food ration supplied

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

daily for each ewe containing 1 kg CFM of 14 % protein plus 1.5 kg good quality alfalfa hay and fresh water was available *ad libitum*.

3. Synchronization protocols

All experiments were starting on a random day which was defined as day 0. Ewes were randomly assigned to seven (6 treatments + 1 control) groups. The animal groups were homogenous according to body condition score, age, and parity. Each animal group was identified with reference color and treated according to the experimental design depicted in Figure 1. Estrus was synchronized using a combination of P4 + eCG or $PGF_{2\alpha}$ + eCG or GnRH + $PGF_{2\alpha}$ hormones as described below.

3.1. Short-term (ST) P4

In (ST) P4 protocol (n = 40), each ewe received 20 mg progesterone acetate in oil [0.8 mL Lutone (Misr, Egypt)] IM day after day for 6 days with 500 IU eCG (Gonaser, Hipra, Spain) on the last day according to Pearce et al.[25] as shown in Figure 1.

3.2. Long-term (LT) P4

In (LT) P4 protocol (n = 40), each ewe received 20 mg progesterone acetate in oil [0.8 mL Lutone (Misr, Egypt)] IM day after day for 12 days as shown in Figure 1, (LT) P4 concurrently with 500 IU eCG (Gonaser) on day 12 according to Hashemi et al.[6]

3.3. Double $PGF_{2\alpha}$, 7 days apart

Each ewe (n = 40) received a double dose of 175 µg $PGF_{2\alpha}$ analog (IM; cloprostenol sodium, Estrumate, Coopers Animal Health Ltd., Berkhamsted, England) 7 days apart[26] with 500 IU eCG (Gonaser) on day 7 as shown in Figure 1.

3.4. Double $PGF_{2\alpha}$, 14 days apart

Each ewe (n = 40) received a double dose of 175 µg $PGF_{2\alpha}$ analog (IM; cloprostenol sodium, Estrumate, England) 14 days apart[27] with 500 IU eCG (Gonaser) on day 14 as shown in Figure 1.

3.5. Traditional Ovsynch

This protocol was applied according to Amiridis et al.[21] where, ewes (n = 40) received (IM) double dose of 8 µg buserelin acetate (Receptal, GnRH agonist, Intervet International, Netherlands) 7 days apart with 175 µg $PGF_{2\alpha}$ analog (Estrumate) on day 5 (Figure 1).

3.6. Non-traditional Ovsynch

This protocol was applied according to Hashem et al.[28] with some modifications where ewes (n = 40) received (IM) double dose of 8 µg buserelin acetate (Receptal) 9 days apart with 175 µg $PGF_{2\alpha}$ analog (Estrumate) on day 7 (Figure 1).

3.7. Control

In the control (n = 40) group each ewe received 1 mL of normal saline on days 0, 7, and 14 without hormonal treatment.

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

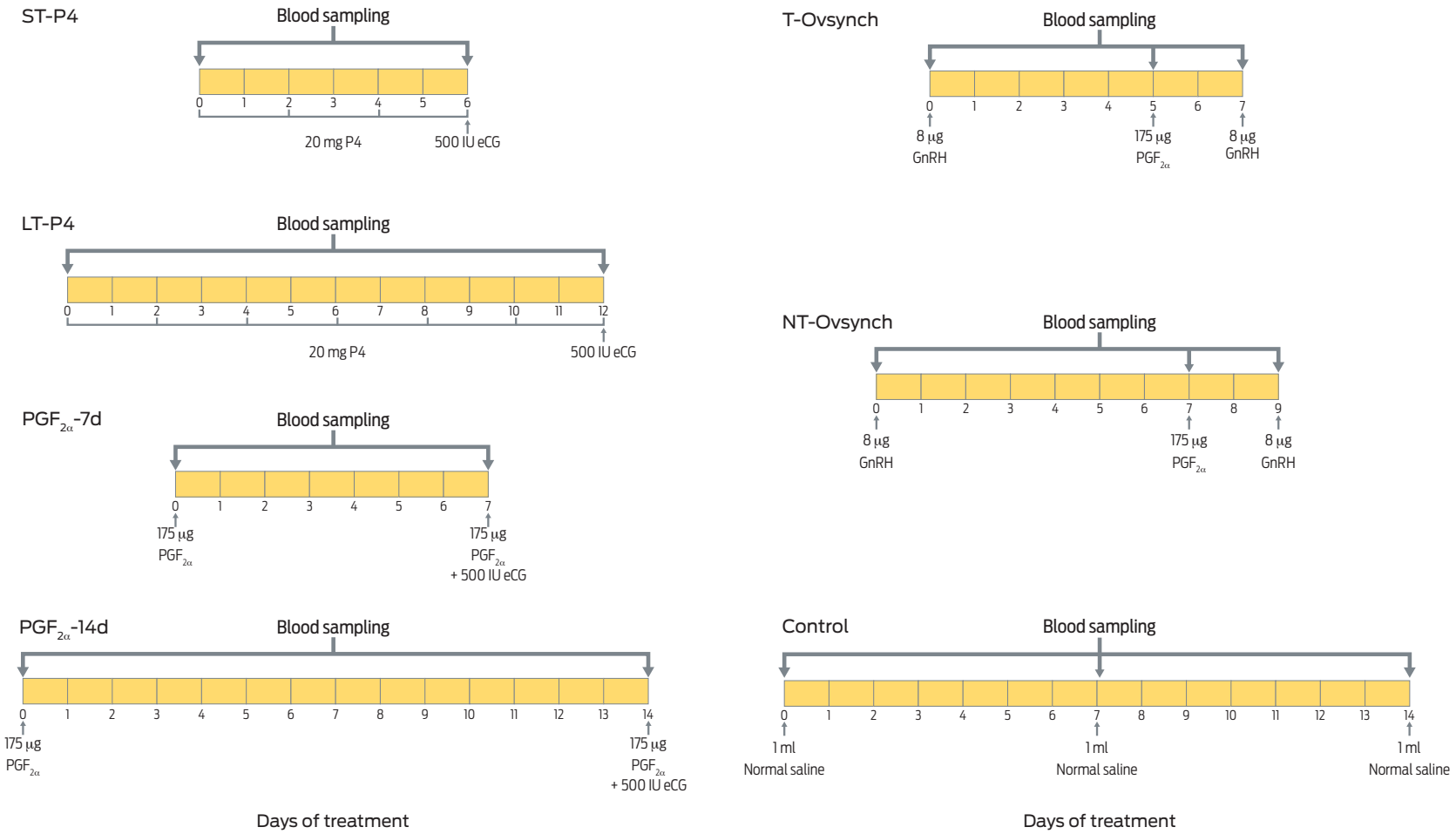

Figure 1. Schematic diagram of estrus and/or ovulation synchronization protocols as well as of control group.
(ST) P4: Treatment by an IM injection of progesterone (P4), all ewes (n = 40) were IM injected with 20 mg P4 acetate (Lutone, Misr, Egypt) day after day for 6 days with 500 IU eCG [equine chorionic gonadotropin (Gonaser, Hipra, Spain)] on the last day. **(LT) P4**: Treatment by an IM injection of P4, all ewes (n = 40) were IM injected with 20 mg P4 acetate (Lutone, Misr, Egypt) day after day for 12 days with 500 IU eCG (Gonaser, Hipra, Spain) on the last day. **$PGF_{2\alpha}$-7d:** Treatment with prostaglandin (PG) $F_{2\alpha}$ was initiated by injection of 175 µg cloprostenol (Estrumate, Coopers Animal Health Ltd., Berkhamsted, England). The second dose of $PGF_{2\alpha}$ was injected 7 days later concurrent with 500 IU eCG. **$PGF_{2\alpha}$-14d:** Treatment with $PGFF_{2\alpha}$ was initiated by injection of 175 µg cloprostenol. The second dose of $PGF_{2\alpha}$ was given 14 days later concurrent with 500 IU eCG. **T-Ovsynch:** Treatment with an initial dose of 8 µg GnRH (Receptal, Intervet International, Netherlands) to synchronize ovulation in a group (n = 40) of ewes. The $PGF_{2\alpha}$was given (IM) 5 days later to lyse the resulting CL. The second GnRH injection was given 2 days after (day 7) $PGF_{2\alpha}$ to increase the synchrony of ovulation. **NT-Ovsynch:** Treatment with 8 µg GnRH to synchronize ovulation in a group (n = 40) of ewes. The $PGFF_{2\alpha}$ was given (IM) 7 days later to lyse the resulting CL. The second GnRH injection was given 2 days after (day 9) $PGFF_{2\alpha}$ to increase the synchrony of ovulation. **Control:** each ewe received (IM) 1 mL of normal saline on days 0, 7, and 14.

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Vol. 10 | 2023

4. Estrus detection and breeding

Immediately after the last treatment, all ewes were carefully observed for estrus signs. Also, fertile Ossimi rams (3–5 years old) of good body condition scores were used to aid in estrus detection twice (morning and evening) daily, one hour each. Estrus detection by using rams (at a ewe:ram ratio of 10:1) was critically employed around the expected time of ovulation. Estrus ewes seek out and tease the ram then nuzzle his belly and finally stand to be mounted at least twice by the ram.[29] After mounting; all ewes were resubmitted to their respective synchronization group.

5. Reproductive efficiency

Estrous activity including estrus rate (number of ewes showing estrus / total number of ewes in each group × 100) and the onset of estrus (appearance of the first positive overt signs of estrus after the last treatment) in hours were calculated for each protocol. Ultrasound (Esaote, Europe BV, Netherlands) scanning of ewes for pregnancy diagnosis was carried out from day 45 to day 50 post-breeding with a transrectal linear (5–10 MHz) probe. Ultrasound scanning was conducted in a standing position. Scanning of the embryonic vesicle with detection of the embryo proper or placentomes were the positive findings of pregnancy. Pregnancy rate (number of pregnant ewes/number of mated ewes), lambing rate (number of ewes which give a live lamb/number of mated ewes), and prolificacy (number of lambs / total number of ewes lambing), as well as gestation period (number of days elapsed from mating until lambing), were calculated.

6. Blood sampling and P4 assay

To detect serum P4 levels among groups during the experiment, blood samples were drawn from the jugular vein into 10 mL coagulant-free centrifuge tubes. At the onset (day 0) of the experiment, blood samples were drawn from all animals (n = 280). On days 5, 6, 9, and 12 blood samples were collected from T-Ovsynch, (ST) P4, NT-Ovsynch, and (LT) P4 groups, respectively. On day 7, samples were collected from $PGF_{2\alpha}$-7d, T–Ovsynch, NT-Ovsynch, and Control groups. Finally, on day 14, jugular blood samples were collected from $PGF_{2\alpha}$-14d and Control groups. The collected samples were allowed to clot at room temperature for exactly an hour before centrifugation at 1 500 × g for 15 min. Sera were harvested into 1.5 mL capacity Eppendorf vials and stored frozen at 30 °C until laboratory analysis. ELISA kits from Biosewoom Inc. (Sungdong-gu, Seoul, Korea, catalog no. BS1405) were used to detect serum P4 levels, with inter and intra-assay coefficients of variation of 2.9 and 4.8 %, respectively. All serum samples for each animal were examined within the same assay and treatments were run in random order.

7. Statistical analysis

The results are presented as the means ± SD. A one-way ANOVA was used to analyze data of the age of ewes, the onset of estrus, the prolificacy, and the gestation period, while Chi-square (χ^2) test was used to analyze estrus rate, pregnancy rate, and lambing rate using the General Linear Model procedure of SAS.[30] Differences

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

among means were tested using the Range Multiple tests of Duncan.[31] Statistical analyses of P4 levels between and among days within the same protocol were determined by Student's two-tailed *t*-test and repeated measures ANOVA, respectively. Differences were considered significant at $P < 0.05$.

Results

1. Estrous activity

Estrus rate was extremely ($P < 0.01$) different among groups and all long-term ((LT) P4, $PGF_{2\alpha}$1-4d, and NT-Ovsynch) synchronization protocols recorded the highest (90%) rate as set out in Table 1. Also, the onset of estrus revealed highly significant ($P < 0.01$) variations among the majority of protocols, where NT-Ovsynch showed the earliest (6.20 ± 0.58 h) onset; whereas it was late (66.50 ± 2.75 h) in (ST) P4 and latest (91.60 ± 4.27 h) in the Control group. It was notable that, all long-term synchronization protocols showed earlier estrus in comparison with their short-term counterparts (Table 1).

2. Reproductive outcomes and gestation period

Results presented in Table 1 revealed that the highest (90%) estrus rate achieved by $PGF_{2\alpha}$-14d protocol yielded the maximum (100%) pregnancy and lambing rates while the lowest (28.57%) pregnancy and lambing rates were recorded in T-Ovsynch protocol. Fortunately, in this study there are no abortions or fetal deaths thereby; the lambing rate was identical to the pregnancy rate for each synchronization protocol (Table 1). The lambing rate was 55.55, 50, 44.44, 33.33, and 0% for (LT) P4, $PGF_{2\alpha}$-7d; NT-Ovsynch; (ST) P4, and Control groups, respectively. Pregnancy and lambing rates, as well as prolificacy of long-term ((LT) P4, $PGF_{2\alpha}$-14d, and NT-Ovsynch) synchronization protocols, were greater ($P < 0.05$) than those of short-term counterparts. Meanwhile, (LT) P4 synchronized ewes had the highest (1.75) prolificacy, and (ST) P4 and T-Ovsynch synchronized ewes had the lowest (1.0) prolificacy as shown in Table 1. The gestation period was significantly shorter ($P < 0.01$) in T-Ovsynch (150.3 ± 1.05) compared to (ST) P4 (153.6 ± 0.34), $PGF_{2\alpha}$-14d (153.5 ± 0.41), and NT-Ovsynch (153.8 ± 0.94) but similar with that of other protocols (Table 1).

3. Progesterone assay

Data shown in Table 2 illustrates the serum P4 levels of treated and control groups. Serum P4 level was greater ($P < 0.01$) in (ST) P4 (2.29 ± 0.15 ng/mL) and (LT) P4 (2.61 ± 0.08 ng/mL) treated ewes on days 6 and 12, respectively as shown in Table 2. Also, serum P4 levels increased ($P < 0.05$) on day 7 and day 14 in $PGF_{2\alpha}$-7d and $PGF_{2\alpha}$-14d, respectively. Furthermore, on the day of $PGF_{2\alpha}$-treatment, the P4 level was extremely ($P < 0.01$) higher than their corresponding values on days of GnRH treatment in either T-Ovsynch or NT-Ovsynch protocols. In the control group, serum P4 level on day 7 was greater ($P < 0.01$; 0.86 ± 0.07 ng/mL) than that on day 14 but similar ($P > 0.05$) to that on day 0 (Table 2).

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Table 1. The reproductive parameters of treated and control Ossimi ewes during the early summer season

Groups[1]	No. of ewes	Age of ewes (month)	No. of estrus ewes	Estrus rate (%)[2]	The onset of estrus (h)[3] (mean ± SD)	Pregnancy rate (%)[4]	Lambing rate[5] (%)	Prolificacy[6]	Gestation period[7]
(ST) P4	40	47.65 ± 1.83a	24	60^{b}	66.50 ± 2.75^{b}	33.33^{cd}	33.33^{cd}	1.00^{d}	153.6 ± 0.34^{a}
(LT) P4	40	51.15 ± 2.24a	36	90^{a}	37.50 ± 1.32^{d}	55.55^{b}	55.55^{b}	1.75^{a}	151.4 ± 0.81^{ab}
$PGF_{2}\alpha$-7d	40	46.15 ± 1.79a	24	60^{b}	55.67 ± 3.47^{c}	50.00^{b}	50.00^{b}	1.33^{c}	153.4 ± 0.27^{ab}
$PGF_{2}\alpha$-14d	40	44.90 ± 1.78a	36	90^{a}	46.25 ± 2.25^{c}	100^{a}	100^{a}	1.65^{a}	153.5 ± 0.41^{a}
T-Ovsynch	40	48.10 ± 2.03a	28	70^{ab}	15.25 ± 3.68^{e}	28.57^{d}	28.57^{d}	1.00^{d}	150.3 ± 1.05^{b}
NT-Ovsynch	40	50.25 ± 1.33a	36	90^{a}	6.20 ± 0.58^{e}	44.44^{bc}	44.44^{bc}	1.50^{b}	153.8 ± 0.94^{a}
Control	40	46.90 ± 2.01a	4	10^{c}	91.60 ± 4.27^{a}	0^{e}	0^{e}	0^{e}	-
P-value	-	0.2260	-	< 0.0010	< 0.0010	< 0.0100	< 0.0100	< 0.0010	< 0.0100

[1]ST-P4 = short-term progesterone, (LT) P4 = long-term progesterone, $PGF_{2}\alpha$-7d = double dose of $PGF_{2}\alpha$, 7 days apart, $PGF_{2}\alpha$-14d = double dose of $PGF_{2}\alpha$, 14 days apart, T-Ovsynch = traditional ovulation synchronization, NT-Ovsynch = non-traditional ovulation synchronization.
[2]Number of ewes exhibiting estrus signs / number of treated ewes × 100.
[3]Hours after the end of hormonal treatment.
[4]Number of pregnant ewes/number of bred ewes × 100.
[5]Number of ewes born a live lamb / number of bred ewes × 100.
[6]Number of lambs born / number of ewes lambing.
[7]Number of days elapsed from breeding until lambing.
[a,b,c,d,e,f] Within the same column values bearing different superscripts in the same column are significantly different ($P < 0.05$).

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Vol. 10 | 2023

Table 2. Serum P4 concentration of treated and control ewes[1]

Sampling day	Groups[2]						
	(ST) P4	(LT) P4	$PGF_{2\alpha}$-7d	$PGF_{2\alpha}$-14d	T-Ovsynch	NT-Ovsynch	Control
0	0.62 ± 0.06[b]	0.63 ± 0.04[b]	0.93 ± 0.11[b*]	1.25 ± 0.17[b*]	0.32 ± 0.10[b]	0.63 ± 0.06[b]	0.66 ± 0.16ab
5	-	-	-	-	1.62 ± 0.09[a]	-	-
6	2.29 ± 0.15[a]	-	-	-	-	-	-
7	-	-	1.59 ± 0.10[a]	-	0.30 ± 0.22[b]	1.87 ± 0.07[a]	0.86 ± 0.07a
9	-	-	-	-	-	0.72 ± 0.03[b]	-
12	-	2.61 ± 0.08[a]			-	-	-
14	-	-	-	2.60 ± 0.43[a]	-	-	0.59 ± 0.03b
P-value	< 0.0010	< 0.0010	< 0.0100	< 0.0500	< 0.0010	< 0.0010	< 0.0100

[1]P4 concentration expressed as ng/mL. P4 concentration (mean ± SD).

[2]ST-P4 = short-term progesterone, (LT) P4 = long-term progesterone, $PGF_{2\alpha}$-7d = double dose of $PGF_{2\alpha}$, 7 days apart, $PGF_{2\alpha}$-14d = double dose of $PGF_{2\alpha}$, 14 days apart, T-Ovsynch = traditional ovulation synchronization, NT-Ovsynch = non-traditional ovulation synchronization.

[a,b]Means bearing different superscripts in the same column are significantly different ($P < 0.05$).

Within the same row means bearing asterisk were significantly different at $P < 0.05$. (ST) P4 = short-term progesterone, (LT) P4 = long-term progesterone, $PGF_{2\alpha}$-7d = double dose of $PGF_{2\alpha}$, 7 days apart, $PGF_{2\alpha}$-14d = double dose of $PGF_{2\alpha}$, 14 days apart, T-Ovsynch = traditional ovulation synchronization, NT-Ovsynch = non-traditional ovulation synchronization.

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Discussion

The obtained results suggest that (LT) P4 is more efficient than (ST) P4 which is consistent with our earlier study that revealed out-of-season Rahmani ewes treated with 20 mg P4 day after day for 12 days had 90 % estrus rate and 44.4 % lambing rate,[16] also, supporting the results of Bartlewski et al.[32] who proven that, administering P4 at the end of diestrus reduces ovulation rate in moderately prolific breeds of sheep which might be the putative reason for their lower lambing rates following their higher estrus rates. However, that leaves unexplained that (LT) P4 yielded greater (1.75) prolificacy in ewes treated with P4 for a longer time (12 days) in comparison with (ST) P4 (1.0). Administering P4 late in the cycle may be a factor, but it cannot be the ultimate cause of this lower ovulation rate.

Since eCG is incorporated in our P4-based protocols, multiple ovulations are anticipated which are usually accompanied by a high incidence of embryonic deaths after day 25 of pregnancy owing to the decrease in uterine capacity.[33] These findings support the concept that the incorporation of eCG in estrus synchronization protocols improves fecundity but not the fertility of ewes.[34] Although, in our (LT) P4 protocol the majority (90 %) of treated ewes exhibiting estrus, only 55.55 % become pregnant and lamb supporting the findings of Husein and Kridli[35] who found that progestagen-eCG based protocol yielded lower pregnancy and lambing rates due to eCG has long half-life which in turn, leads to the development of large persistent follicle that adversely affect the early embryonic development and/or embryo transport from the uterine tube to the uterine horn.

Doubtless, $PGF_{2\alpha}$ and GnRH agonists were successful to induce acceptable values of estrus synchrony and reproductive outcome after natural breeding.[36] Moreover, when compared with intravaginal progestagens, such protocols are feasible, simple, and practical because they involve an IM injection only, and they are not invasive as well. From the physiological point of view, the effect of $PGF_{2\alpha}$ on multiple ovulation and prolificacy remains equivocal with a variable effect on the ovulation rate.[11] Moreover, considering the seasonal nature of the ewe estrous cycle, $PGF_{2\alpha}$ is only effective in animals already cycling. Suggesting that, $PGF_{2\alpha}$ is employed for estrus synchronization during the breeding season only in seasonal breeder ewes, according to the luteolytic effect of the hormone.[37] In the current study, as expected, some ewes had functional CL (P4 level $>$ 1 ng/mL) during the early summer season and responded to $PGF_{2\alpha}$-based protocols during this transition period.

In our study, a greater proportion of ewes responded to either short ($PGF_{2\alpha}$-7d) or long ($PGFF_{2\alpha}$-14d) interval double $PGF_{2\alpha}$ protocols during the early summer season which emphasizes the results of Contreras-Solís et al.[38] who proposed that $PGF_{2\alpha}$could be used for estrus synchrony all year round in sheep farms located in the tropic. Additionally, in the subtropics, ewes are almost cyclic all year round due to the season mainly affecting the luteal functions with minor influences on follicular development.[39] Further, in the current study, all ewes were more than 44 months old where the seasonality of breeding is less obvious compared with young ewes.[40]

Our results evidenced that Ossimi ewes are cyclic throughout the year which could be attributed to the subtropical location of Egypt plus proper nutrition and the presence of rams within the herd. Moreover, $PGF_{2\alpha}$-14d was successful in inducing 100 % fertile estrus combined with full reproductive success in ewes during

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

the early summer season, supporting the concept that, long-interval double $PGF_{2\alpha}$ protocols were efficacious in inducing fertile estrus in comparison with short-interval double $PGF_{2\alpha}$ protocols.[13,14] Further, $PGF_{2\alpha}$ induces luteolysis which includes functional and structural regression of CL[41] with a decrease in serum P4 level.[42] These findings might be a reasonable explanation for this greater reproductive outcome obtained with the $PGF_{2\alpha}$-14d protocol.

Herein, long-term synchronization protocols coupled with eCG ((LT) P4 and $PGF_{2\alpha}$-14d) showed a higher estrus rate than that of short-term ((ST) P4 and $PGF_{2\alpha}$-7d) counterparts which is consistent with the previous study that showed higher estrus response after treatment with P4 in association with gonadotropins.[43] Another important feature of these long-term protocols was their greater prolificacy compared with their short-term counterparts. This finding could be attributed to prolonged exposure of ewe ovaries to P4 leading to the proper selection of dominant follicles,[44] resulting in a larger CL, capable to maintain the pregnancy until full-term.[45] This assumption is supported by the obtained higher serum P4 levels at the end of (LT) P4 (2.61 ± 0.08 ng/mL) and $PGF_{2\alpha}$-14d (2.60 ± 0.43 ng/mL) protocols.

The most significant finding of this study is that $PGF_{2\alpha}$-14d yielded a greater estrus rate concurrent with the maximum pregnancy and lambing rates which can be attributed to its better hormonal profile in comparison with $PGF_{2\alpha}$-7d.[12] Long intervals between $PGF_{2\alpha}$ injections result in longer periods of higher plasma P4 levels before mating, higher plasma oestradiol levels around the onset of estrus,[12] and also, a higher conception rate.[46] This notion has been confirmed by Fierro et al.[13] who found that long-interval double $PGF_{2\alpha}$ yielded a greater proportion of conceived ewes following cervical insemination with fresh semen. On the other hand, in short intervals, there are altered plasma P4 levels at the end of the luteal phase which may impair the pre-ovulatory actions and the success of conception.[13] Furthermore, our results are in accord with Besufkad et al.[14] who found that double $PGF_{2\alpha}$, 11 days apart, yielded a greater conception rate and prolificacy when compared with double $PGF_{2\alpha}$, 7 days apart.

Our on-farm trial revealed that T-Ovsynch slightly improves the reproductive performance of ewes during the early summer season. This is likely due to some of T-Ovsynch treated ewes having been shown a false heat or exhibited a true heat before $PGF_{2\alpha}$ according to Titi et al.[47] This could be the putative causative factor because P4 levels on the day of $PGF_{2\alpha}$ treatment in either T-Ovsynch or NT-Ovsynch were greater than their counterparts on the days of GnRH, suggesting the success of the first GnRH to induce ovulation with subsequent CL formation. Furthermore, the animal breed, type, and dose of the used hormones as well as the season of the year might be responsible for this low response because Ashmawy[48] found that, the application of T-Ovsynch during the breeding season was successful to improve Rahmani ewe fertility. Also, the Control group showed only 10% of infertile estrus and failed to respond to the ram effect.

Another possible reason for this low reproductive outcome of Ovsynch protocols is the short half-life of the used GnRH analog (buserelin) in comparison with the long half-life of eCG as reported by Luther et al.[49] Despite, herein, we duplicate the recommended dose of GnRH analog (8 µg buserelin); it resulted in a lower (28.57%) lambing rate and minimum (1.00) prolificacy. This might be due to the absence of either eCG or a source of P4 in our T-Ovsynch protocol because

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Martinez et al.[22] found that a combination of GnRH, P4, $PGF_{2\alpha}$ and eCG was effective to induce estrus and ovulation in anestrus ewes.

Although obvious numerical differences existed in the values of estrus rate and the onset of estrus between T-Ovsynch and NT-Ovsynch, none of the values varied statistically ($P>0.05$). On contrary, NT-Ovsynch yielded greater pregnancy and lambing rates as well as prolificacy compared with T-Ovsynch. This might be attributed to the prolonged exposure of the growing follicles to P4 in NT-Ovsynch (7 days) in comparison with T-Ovsynch (5 days). Ewes when induced to ovulate during anestrus, it frequently develops a short-lived (3–4 days) CL associated with to lack of the previous P4.[50] This short-lived CL might be implicated in the obtained low pregnancy and lambing rates following the higher estrus rate of both T-Ovsynch and NT-Ovsynch. Unfortunately, we did not evaluate the P4 level in these Ovsynch protocols after breeding to exclude this short-lived CL.

In another word, Ovsynch protocols induce a short estrous cycle[51] owing to the second GnRH, inducing ovulation of follicles that had not enough time to get final maturation which might be a plausible explanation for the obtained low pregnancy and lambing rates following the higher estrus rate of both T-Ovsynch and NT-Ovsynch protocols. It is well known that Ovsynch protocols are originally designed to induce ovulation, but not estrus, and to apply fixed-timed insemination.[28] Unfortunately, we have not frozen semen and thus, our Ovsynch protocols were estrus detection-based followed by natural mating. Nevertheless, successful induction of ovulation in ewes during the early summer season would provide the possibility to give lambs all over the year and thereby increase ewe's lifetime productivity.

Conclusions

Although all treatments had a positive effect on the reproductive performance of Ossimi ewes during the early summer season; long-term synchronization protocols, particularly double $PGF_{2\alpha}$-14 days apart, had a better effect on ewe fertility when compared with short-term protocols. Also, this study has proven that Ossimi ewes are cyclic during the early summer season. The present findings pave the way to design simple and practical methods to maximize the reproductive outcome of ewes at least of the Ossimi breed.

DOI: http://dx.doi.org/10.22201/fmvz.24486760e.2023.1058
Vol. 10 | 2023

Data availability

All relevant data are within the manuscript and its supporting information files.

Acknowledgments

The authors thank the staff of Riwina Animal Production station and the Ethics Committee of Animal Experimentation of Kafrelsheikh University, Faculty of Veterinary Medicine for their kind cooperation to achieve the experiments.

Funding statement

This study was not funded by any governmental, private, or non-profit funding agencies.

Conflicts of interest

The authors have declared that there is no conflict of interest concerning this study.

Author contributions

Conceptualization, methodology, investigation, and data curation EAA, FMS, WBE, AR, MS, and FF.

FMS, WBE, and AR perform the statistical analysis being supervised by EAA.

Writing, review, and editing of the manuscript, supervision, formal analysis, and visualization were performed by EAA, FMS, WBE, AR, MS, and FF.

All authors read and approved the final manuscript.

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Vol. 10 ▮ 2023

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