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EFFECT OF PROGESTERONE AND PROSTAGLANDIN ANALOGUE-BASED SYNCHRONIZATION PROGRAMS ON THE FOLLICULAR DYNAMICS AND CONCEPTION RATE AT TWO DIFFERENT BREEDING SEASONS IN SUBTROPICAL EWES

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ABSTRACT

The present study aimed to investigate the effect of using progesterone- and prostaglandin analogue-based synchronization programs on follicular dynamics and conception rate in subtropical ewes during winter and spring breeding seasons. A total of 80 ewes were assigned into 4 equal groups; Sponge-Spring (SS), Sponge-Winter (SW), $PGF_2\alpha$-Spring (PS) and $PGF_2\alpha$-Winter (PW). In $PGF_2\alpha$-based protocols, 2 injections of natural $PGF_2\alpha$ with10 days apart were used. In progesterone-based protocol, one intravaginal progestagen impregnated sponge inserted for 14 days. The estrus behavior was detected every 4 hrs after the second injection of $PGF_2\alpha$ or the withdrawal of sponge (Day 0) until the estrus signs were no longer detected using teaser rams. Mating with fertile rams was scheduled every 12 hours during heat. Ultrasonography examinations were started at day 0 and every 12 hrs for 5 days. The results showed that season significantly affected the duration of estrus ($P < 0.05$) which was longer in winter than spring (SW; 29.6 ± 3.0, PW; 27.1 ± 1.8 Vs. SS; 21.7 ± 2.0 PS; 16.3 ± 3.0 hrs), while the onset of estrus started at the same times in all groups. On the other hand, the treatment manipulated the patterns of follicular growth. In sponge groups, the pattern was classified according to the number of ovulatory follicles (single or multiple) while in $PGF_2\alpha$ groups the classification was according to the origin of follicular wave (before or after second $PGF_2\alpha$ treatment). We found that 22% of dominant follicles were ovulated within 12 hours after injection of PGF2α. The study revealed that season and treatment affected the conception rate and litter size. The spring season significantly decreased the conception rate ($P<0.05$) regardless of the treatment, while sponge increased the litter size ($P<0.001$) in comparison to $PGF_2\alpha$. It was concluded that treatment and season had significant effect on the efficacy of estrus synchronization programs in SAIDI ewes. Using progesterone based programs in winter season increased the conception rate and litter size in comparison to $PGF_2\alpha$ programs.

Keyword: Ewes, PGF2α, sponge, synchronization, ultrasonography.

INTRODUCTION

Sheep are one of the most prominent seasonal breeders among the domestic animals. Modern sheep husbandry is improving the efficiency of extensive production and is controlling the reproductive process for intensive production (Ozyurtlu *et al.,* 2010). Reproductive activity in sheep varies according to the seasonal changes (Gibson and Robinson, 1971; Robinson, 1971), length of the photoperiod (Dogan and Nur, 2006; Lindsay, 1991; O'Callaghan *et al.,* 1992), breed, age, nutritional conditions (Pearse *et al.,* 1994), stress (Daley *et al.,* 1999) environment and male factors (Rosa and Bryant, 2002).

Estrous synchronization is important for improving reproductive performance in sheep and has been accomplished using several methods with variant degree of success (Scaramuzzi and Martin, 1984).

Progesterone based protocols are commonly used worldwide and administered by several methods, routes and doses. The most common route of application is intravaginal sponge (Greyling and van der Nest, 2000; Karaca *et al.*, 2009) and the controlled internal drug releasing device (CIDR) (Dixon *et al.*, 2006; Godfrey *et al.*, 1997; Van Cleeff *et al.*, 1998). It is known that adequate systemic progesterone concentration is essential for developing and functioning the ovarian follicles. The basis to give intravaginal sponge is to decrease LH secretion (Goodman and Karsch, 1980), which suppress estrus, LH surge and ovulation until the sponge is removed. Maintenance of high progesterone and low LH concentration increased the follicle turnover (Leyva *et al.*, 1998; Noel *et al.*, 1994); for that, at the end of treatment, preovulatory follicles are expected to be in the growing phase and competent to release oocyte. Sometimes, the release of progesterone from sponge is too low, particularly at the end of the treatment. So, LH is not adequately suppressed (Johnson *et al.*, 1996; Vinoles *et al.*, 1999). Thus leads to abnormal follicular development (Menchaca and Rubianes, 2004), which decrease fertility (Allison and Robinson, 1970; Vinoles *et al.*, 2001).

Prostaglandin $F_2\alpha$ analogues are commonly used for estrus synchronization and ovulation induction in ewes (Liu *et al.*, 2006). In ewes, $PGF_2\alpha$ causes regression of the corpus luteum (CL) between days 3 and 13 of the cycle (Rubianes *et al.*, 2003; Titi *et al.*, 2010). $PGF_2\alpha$ is not effective on young CL between D0 -D2 or days 14-17, because the CL is undergoing spontaneous luteolysis. To synchronize estrus and ovulation, double injections of $PGF_{2\alpha}$ were given from 9-11d apart (Liu *et al.*, 2006).

In the view of these considerations, the objective of the current study was to characterize and compare ovarian follicular development by assessing follicular dynamics (ultrasonography), as well as ovulation rate and fertility rate in subtropical ewes synchronized by $PGF_2\alpha$ and commercial intravaginal sponge during two different breeding seasons.

MATERIALS and METHODS

1- Animals and experimental design:

The experiment was carried out during 2 breeding seasons, winter (January-March) and spring (April-May) in Mallawi sheep station, Animal production research institute, Egypt, (latitude 28°07´N and 30°33´E). A total of 80 SAIDI ewes, 3-5 years, 30-40 Kgs body weight were assigned for the present study and maintained outdoor with free access to water.

Ewes were assigned into 4 equal groups (20 ewes for each)); Sponge-Spring (SS), Sponge-Winter (SW), $PGF_2\alpha$-Spring (PS) and $PGF_2\alpha$-Winter (PW). For ewes synchronized with $PGF_2\alpha$, two I.M injections of 2.5 mg of Dinoprost (Lutalyse, Pfizer manufacturing, Purts, Belgium), with 10 days interval were used. For ewes synchronized with progesterone sponge, one intravaginal Progestagen impregnated sponge (40mg fluorogestone acetate, GFA, Chronogest®, Intervet, International, boxmeer, Netherland) was inserted and left for 14 days. After withdrawal of sponge or second injection of $PGF_2\alpha$ estrus behavior was detected every 4 h interval and for 5 days using teaser rams (n=10). Commencement of the estrus was defined as the time when the ewe first stood to be mounted by the ram. Duration of estrus was defined as the interval between the onset and end of estrus signs. The end of estrus was the time when the ewe refused to be further mounted. The mating was scheduled every 12 hours until ewes refused to be mounted by rams. The day of 2^{nd} injection of $PGF_2\alpha$ or Progestagen withdrawal considered as Day 0. Ultrasonography examination was carried out twice daily starting at day 0 and every 12 hrs for 5 days.

2- Monitoring of follicular development:

Ultrasound scanning was performed by the same operator every 12 hrs for 5 days starting at day 0 using an ultrasound scanner (Pie Medical, 100LC, Maastricht, Netherlands) equipped with a changeable 6/8 MHz linear transducer. The examinations were conducted with the ewe in standing position. The urinary bladder used as a guide to locate the uterine horn. The probe was rotated laterally 90° clockwise and 180° anticlockwise to scan the ovaries and genitalia. The size and number of all follicles ≥ 2mm were recorded in retrospective ovarian sketches to determine the topography and the dimensions of each follicle. The follicular dynamic and ovulation rate were recorded while the number of viable embryos was assessed after 25 days.

3- Statistical analysis:

The onset of estrus, duration, end of heat and time of ovulation were statistically analyzed using ANOVA (JMP statistical software version 5.1; SAS Institute, Cary, NC, USA 2002 was used). The mean diameter of the ovulatory follicles and follicular population were compared between groups using t-test. The reproductive performance was analyzed using the Chi-square test. The level of significance was set at $P<0.05$.

RESULTS

1- Characteristics of estrus:

Response for estrus induction, time to onset of estrus and duration of estrus were presented in Table 1. The results show that all ewes in this study came on heat on the same time while the duration of estrus was affected by season. The duration of estrus increased significantly ($P < 0.05$) in winter in comparison to spring season regardless the treatment. In fact, the estrus duration of PS group was the shortest among the groups showing an early follicle ovulation.

2- Follicular dynamics:

The results showed that season has no effect on treatments with regard to the patterns of follicular growth. In sponge groups, the animals showed 2 different patterns according to the number of ovulatory follicles (single or multiple as in Fig 1, a-d). In PG- groups, the follicular growth was classified according to the origin of the follicular wave, before or after second injection of $PGF_2\alpha$ (Fig.2a-d). Further, in the PG-groups, 22% of the D.F. was ovulated 12h after the second injection of $PGF_2\alpha$ without estrus signs or even formation of CL (Fig. 2c).

The size of ovulatory follicle in prostaglandin-synchronized ewes was the smallest ($P<0.05$) among the groups while the number of recruited follicles of SW group was the highest ($P<0.05$) (Table 2).

3- Fertility and fecundity:

The results of fertility and fecundity were presented in Table 3. Fertility rate in spring was significantly ($P<0.05$) the lowest among the groups regardless to the treatment while the sponge groups had the greatest litter size ($P<0.001$). On the other hand the rate of triple fetuses did not differ between groups.

Table 1: The effect of season and treatment on the time of onset, duration of estrus and time of ovulation.

Groups	Onset of heat (h)	Duration of heat (h)	End of heat (h)	Time of ovulation (h)
SS	45.7 ± 2.9	21.7 ± 2^{bc}	67.4 ± 3.3^{a}	59 ± 4.0^{a}
SW	46.9 ±3.7	29.6 ± 3.0^{a}	76.5 ± 2.2^{a}	70.7 ± 3.7^{a}
PS	37.2 ± 5.3	16.3 ± 3.0^{c}	53.5 ± 5.6^{b}	39 ± 6.5^{b}
PW	47.4 ± 1.4	27.1 ± 1.8^{ab}	74.5 ± 1.9^{a}	67.1 ± 8.7^{a}

The values with different superscripts (a,b,c) in the same column differ significantly ($P<0.05$). Where sponge spring (SS), sponge winter(SW), Prostaglandin spring (PS) and Prostaglandin winter (PW).

Table 2: The effect of season and treatment on the size of the ovulatory follicle and the number of recruited follicles.

	SS (n=10)	SW (n=10)	PS (n=10)	PW (n=9)
Ovulatory Follicle (mm)	6.1 ± 0.2^{ab}	6.1 ± 0.3^{ab}	5.7 ± 0.3^{b}	6.7 ± 0.3^{a}
Recruited follicles	6.2 ± 0.6^{b}	8.7 ± 0.7^{a}	5.7 ± 0.6^{b}	5.6 ± 0.6^{b}

The values with different superscripts (a,b) in the same column differ significantly ($P<0.05$). Where sponge spring (SS), sponge winter (SW), Prostaglandin spring (PS) and Prostaglandin winter (PW).

Table 3: The effect of season and treatment on the fertility, lambing and twining rate.

%	Fertility rate	Fecundity rate	Single fetus	Double fetus	Triple fetus
SS (n=20)	45% (9/20)[b]	77.8% (7/9)[a]	22.2% (2/9)	66.7% (6/9)	11.1% (1/9)
SW (n=19)	73.7% (14/19)[a]	50% (7/14)[ab]	50% (7/14)	42.9% (6/14)	7.1% (1/14)
PS (n=21)	52.3% (11/21)[b]	36.4% (4/11)[b]	63.6% (7/11)	27.3% (3/11)	9.1% (1/11)
PW (n=19)	73.7% (14/19)[a]	7.1% (1/14)[b]	92.9% (13/14)	0	7.1% (1/14)

The values with different superscripts (a,b) in the same column differ significantly ($P<0.05$). Where sponge spring (SS), sponge winter(SW), Prostaglandin spring (PS) and Prostaglandin winter (PW)

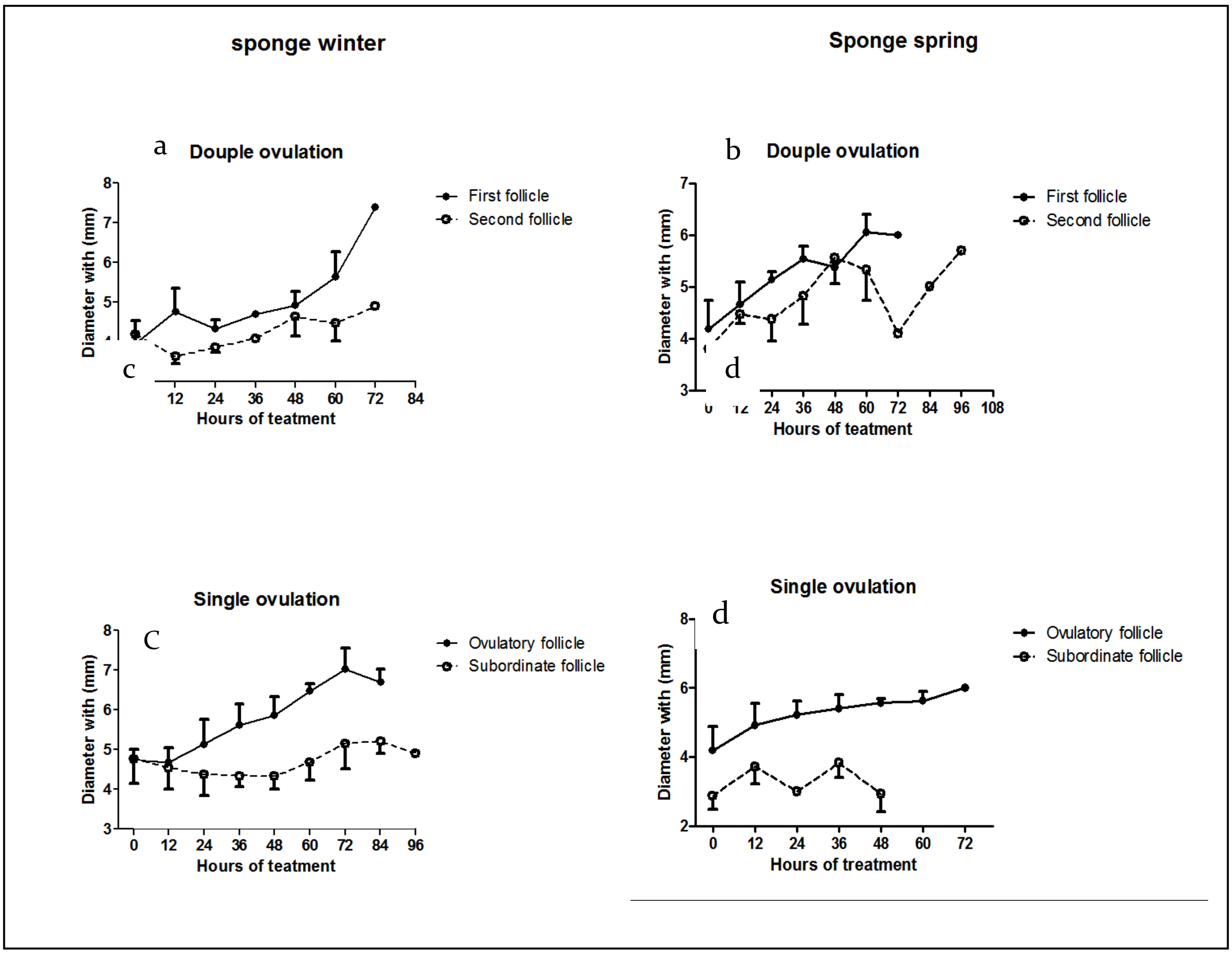

Fig. 1: Mean diameter of the ovulatory follicles. (a,b) Mean (+SEM) diameter of the first and second ovulatory follicles in the double ovulation animals. (c,d) Mean (+SEM) diameter of the ovulatory and subordinate follicle in the single ovulation animals.

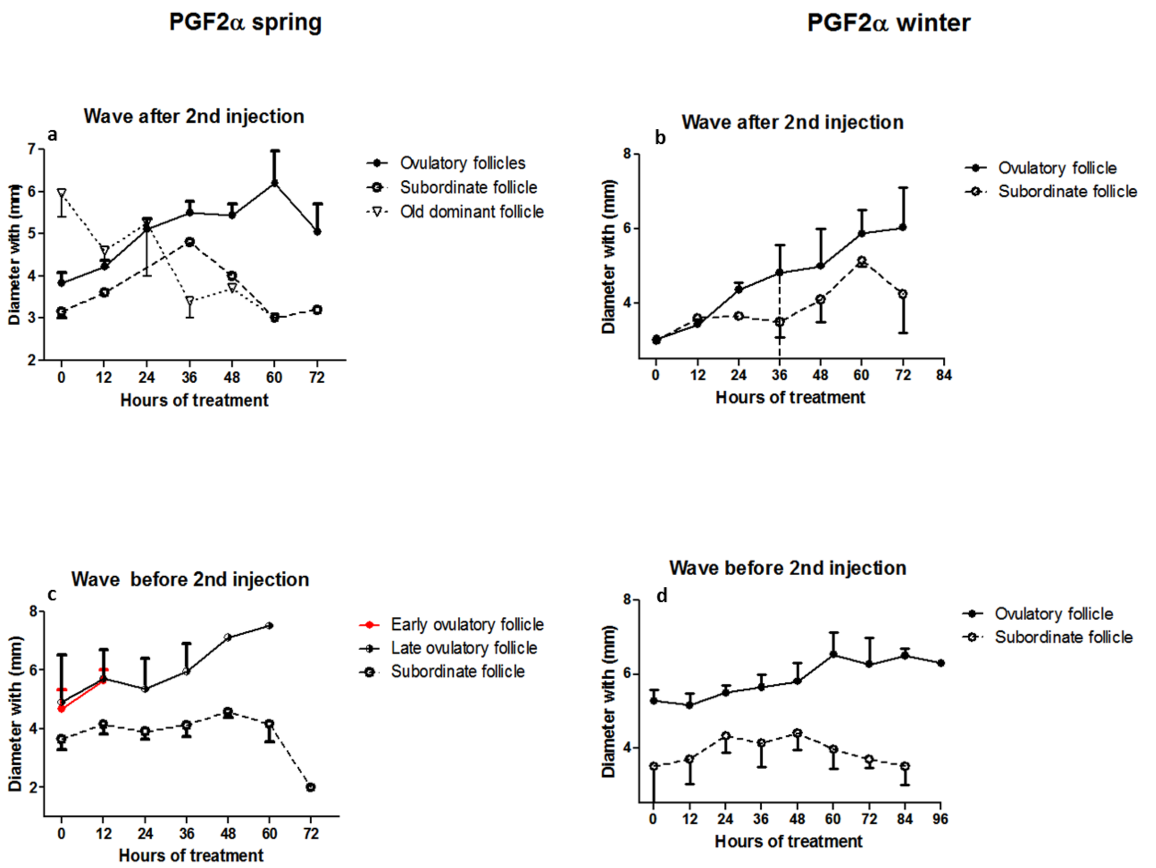

Fig. 2: Mean (±SEM) diameter of the ovulatory follicles recruited from waves after 2nd $PGF_2\alpha$ injection (a, b). Mean (±SEM) diameter of the ovulatory follicles recruited from wave before 2nd $PGF_2\alpha$ injection (c, d).

DISCUSSION

Onset of heat did not significantly affected by either season or treatment although PS group showed the earliest onset of estrus (37.2±5.3 hrs). The onset of heat after using $PGF_2\alpha$ and progesterone in previous studies was controversial. In Ethiopian Menze sheep, Progestagen-synchronized ewes exhibit estrus later than the prostaglandin-synchronized ewes (Mutiga and Mukasa-Mugerwa, 1992). Several reports confirming that exogenous progesterone lessened the number of short estrus and delayed the onset of heat from 20.5 to 58.8 hrs (lassoued *et al.*, 1995) with an average of 48 h after the sponge withdrawal (Zarkawi, 2001). However, in tropical PG synchronized ewes, the onset of estrus occurred within 31.6 ± 2.3 hrs which was longer than of sponge synchronized ewes (25.4 ± 2.3hrs) (Godfrey *et al.*, 1999). In the present study, the male might play a role in the obtained results. The continual exposure to a buck following synchronized estrus by either cloprostenol (PG) or intravaginal sponge shortened the interval to estrus in Nubian does from 48 to 38 hrs (Romano, 1993). Further evidence showed that when the buck served the does the onset of estrus was shortened (Romano, 1994). Such response was mediated through the mechanical action of the penis against the vagina (Romano, 1994).

The duration of heat was significantly ($P<0.05$) longer in winter either in sponge or $PGF_2\alpha$ (29.6±3.0 & 27.1±1.8hrs) than spring. It was reported that higher temperature during spring season reduce the duration of heat in ewes (Igono *et al.*, 1982) and tropical does (Cerbito *et al.*, 1995). On the other hand, the treatment did not affect the onset of ovulation between groups. The same result was reported by (Godfrey *et al.*, 1999).

The result showed that follicular wave pattern of PG-synchronized ewes did not affect the ovulation. In another species like western white face ewes, 42% of ovulated follicle was emerged from waves originated after 2nd dose of PG (Liu *et al.*, 2006). It seems that ill-timed luteolysis after PG treatment might alter the recruitment of follicles for ovulation in ewes (Barrett *et al.*, 2002). In fact, even if PG has been injected to induce luteolysis around the expected time of follicle wave emergence, it will not prevent the variations in the source of ovulatory follicles (Liu *et al.*, 2006). In comparison to cattle, giving PG at follicle wave emergence consistently resulted in the ovulation of a follicle from new follicular wave (Duggavathi *et al.,* 2004).

Breakthrough ovulation was observed in 22% of ewes within 12 hrs after 2^{nd} injection of $PGF_2\alpha$. These ovulations were preceded without signs of heat or formation of CL. The same observation was reported in Western white face ewes (Davies *et al.,* 2006; Ford *et al.,* 1977) suggesting that PG has a direct stimulatory effect on large follicle. Another evidence regarding how $PGF_2\alpha$ has a direct ovulatory effect on the follicle comes from study of the bovine ovarian arterial smooth muscle. $PGF_2\alpha$ reduced the follicular blood flow by vasoconstriction of the blood vessels at the rupture point during the later portion of the ovulation (Ford *et al.,* 1977).

The removal of the sponge leads to a decline in the progesterone concentration, which leads to a preovulatory LH surge and the cascade of events that results in ovulation of follicle (Niswender *et al.,* 1986). Sponge inhibits the ovarian cycle and consequently ovulation (Al-Merestani *et al.,* 2003) and so control the time of follicular ovulation.

Winter ovulatory follicles were larger than spring follicles in PG-synchronized ewes. The short day photoperiod delays the release of the LH in comparison to the long day photoperiod (Skinner and Herbison, 1997). Such delay was concomitant with increase in follicular size. In sponge groups, there was no significant different between the size of the ovulatory follicles. The basis of administering progesterone is to decrease LH secretion (Goodman and Karsch, 1980) which prevents the estrus LH surge and ovulation until the withdrawal of the sponge. In contrary to our results, Progestagen resulted in larger follicles in comparison to the PGF_2a in Manchega sheep (Gonzalez-Bulnes *et al.,* 2005). The contradiction between the two studies may be related to the breed and season differences.

The number of recruited follicles was significantly higher in SW group (8.7±0.7) than other groups. Similarly, the number of small follicles was significantly high in winter season in Ossimi ewes in the subtopics (Ali *et al.,* 2006). In another species, Suffolk ewes, the seasons did not affect the number of follicles (Noel *et al.,* 1993). It was reported that number of small follicles increased with $PGF_2\alpha$ and decreased with Progestagen (Gonzalez-Bulnes *et al.,* 2005).

The results indicated that season not treatment had a significant effect on the fertility of ewes. It was reported that the twining and fertility rate increased in winter than summer and fall (Huston, 1983). Using a compination between sponge and PG together or with equine chorionic gonadotrophin (eCG) increased the fertility rate more than using PG or sponge alone. For example, sponge and eCG had a 100% fertility rate compared to 37% for double injections of $PGF_2\alpha$ in Karakul ewes (Safdarian *et al.,* 2006). Difference may be attributed to the use of eCG which induce ovulation. Moreover, the combination between $PGF_2\alpha$ and sponge improved the conception rate better than using $PGF_2\alpha$ alone (Loubsera and van Niekerka, 1981).

The presnt study showed that fecundity rate was affected by the treatment not the season. The fecundity rate increased significanly within the sponge groups in comparison to the $PGF_2\alpha$ groups. This may be attributed to the increased number of growing follicles associated with the usage of sponge and the higher number of double ovulation in synchronized ewes. In Awassi ewes, treatment with progeterone sponge increased the twinning rate by 20% (Zarkawi, 2001).

In conclusion, Synchronization of estrus in ewes with progesterone sponge during winter season had the highest fertility, litter size and proven to be superior over PG or synchronization during spring season in subtropics.

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تأثير برامج التزامن باستخدام البروجيستيرون ومثيل البروستاجلاندين على ديناميكيات الجريبات ومعدل الحمل اثناء موسمين مختلفين للتكاثر فى النعاج شبه الاستوائية

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تهدف هذه الدراسة إلى معرفة أثر استخدام هرمون البروجسترون والبروستاجلاندين فى برامج التزامن لدوره الشبق واثره على ديناميكيات الجريبات ومعدل الحمل في النعاج شبه الاستوائية خلال فصل الشتاء ومواسم التكاثر الربيعي. تم تقسيم 80 نعجه إلى 4 مجموعات متساوية؛ الإسفنج فى الربيع (SS)، الإسفنج فى الشتاء (SW)، والبروستاجلاندين فى الربيع (PS) والبروستاجلاندين فى الشتاء (PW). في بروتوكول البروستاجلاندين: تم حقن النعاج بعدد حقنتين في بروتوكول البروجسترون بفارق 10 أيام: ووضع اسفنجه مهبليه مشربة بالبروجيستيرون فى بروتوكول البروجيستيرون لمدة 14 يوما. تم الكشف عن سلوك الشبق باستخدام كباش كشافه كل اربعه ساعات بعد الحقنة الثانية من البروستاجلاندين أو سحب الإسفنجه واعتباره اليوم صفر من الفحص حتى انتهاء علامات الشبق. وتم التزاوج مع كباش خصبة كل 12 ساعة خلال الشبق. وبدأ الفحص في اليوم 0 باستخدام جهاز الموجات فوق الصوتية كل 12 ساعة لمدة 5 أيام. أظهرت النتائج أن الموسم له تأثيرا ملموسا على مدة الشبق (P <0.05) والتي كانت تعد في فصل الشتاء اطول من فصل الربيع (SW؛ 3.0 ± 29.6، PW، 1.8 ± 27.1 مقابل SS؛ 2 ± 21.7، PS، 3.0 ± 16.3 ساعة) ، في حين بدأ ظهور الشبق في الأوقات نفسها في جميع الفئات. من ناحية أخرى، فإن العلاج غير أنماط النمو الجريبي. ففي مجموعات الإسفنج، صنفت نمطيا وفقا لعدد جريبات التبويض (الى جريبه واحده فى الموجه واكثر من جريبه)، في حين انه في مجموعات البروستاجلاندين صنفت وفقا للموجة التى ظهرت منها الجريبه (موجه ظهرت قبل أو بعد الحقن بالحقنه الثانية). وجد أن 22٪ من الجريبات المهيمنة قد حدث لها تبويض في غضون 12 ساعة بعد الحقن من البروستاجلاندين. وكشفت الدراسة أن كل من الموسم والعلاج كان له تاثير على معدل الحمل وعدد الاجنه. ففى موسم الربيع انخفض بشكل ملحوظ معدل الحمل (P <0.05) بغض النظر عن العلاج، في حين زاد عدد الاجنه فى مجموعات الاسفنج (P <0.001) بالمقارنة مع البروستاجلاندين. وخلصت الدراسه إلى أن العلاج بالبروستاجلاندين والاسفنجه والمواسم كان له تأثير كبير على فاعلية برامج التزامن في شبق النعاج الصعيدى. وان استخدام برامج البروجسترون في فصل الشتاء ادى الى ارتفاع معدل الحمل وعدد الاجنه مقارنه ببرامج البروستاجلاندين.