



EFFECT OF DIFFERENT REPRODUCTIVE PROTOCOLS OF ESTRUS SYNCHRONIZATION ON ESTROUS SIGNS APPEARANCE RATE FOR AWASSI AND NAIMI SHEEP BREEDS.

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ABSTRACT

The experiment was conducted at the Khairat Al-Ittihad station located in the Al-Shumali district of Babil Governorate from January 1, 2024, to June 20, 2024, to assess the impact of certain hormonal treatments on the reproductive efficiency parameters of Awassi and Naimi ewes Including local production and required 2-3 lambs per year from the ewe to load. Twenty-four Awassi sheep and twenty-four Naimi sheep were selected and divided into eight groups, with six sheep in each group (four groups of Awassi sheep and four groups of Naimi sheep). Results showed a significant effect of time (period) of protocols or sponges for 9 days and after removal, 125 micrograms of Prostaglandin (PGF2 α) hormone and 330 international units of Equine chorionic gonadotrophin (eCG) hormone were injected on estrous signs appearance in Awassi ewes ($P \leq 0.05$), the best signs were recorded after 48 hours from treatment (50%) and about 33.33% of estrus signs were noticed after 72 hours and no estrus signs were seen after 96 hours after treatment. The sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of Gonadotropin-Releasing Hormone (GnRH) were injected (treatment 2) effected significantly ($P \leq 0.01$) on estrous signs appearance. A significant difference among the ewes that treated with 4th treatment which including Controlled Internal Drug Releasing Device (CIDR) for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected, the best estrus signs rate was recorded after 48 hours from treatment while the rates were 16.67, 16.67 and 0.00% after 24, 72 and 96 hours of treatment respectively. Results showed a significant differences in the appearance rate of estrus signs of Naimi sheep breed that treated with different reproductive treatments in different times ,the ewes that treated with sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected differed significantly ($P \leq 0.05$) in the time of estrous signs appearance, the highest rate was noticed at 48 hours after treatment (50.0%) while the lowest rate of signs was recorded at 96 hours after treatment (0.0%). strus signs appearance rate differed significantly ($P \leq 0.05$) among ewes that treated with different protocols after 48 hours of treatment, the highest rate was noticed in ewes with 2nd treatment namely, 50.00% while the rates was 33.33, 33.33 and 0.00 % in ewes that treated with 1st, 3rd and 4th treatments respectively. the pregnancy rate in Awassi and Naimiya ewes was 100% and 91% respectively.

Keywords: Awassi and Naimi sheep, estrus signs, hormones, 1 sponges.

*This article is taken from the doctoral dissertation of the first researcher.



تأثير البروتوكولات التناسلية المختلفة لتزامن الشبق على معدل ظهور علامات الشبق لسلالات الأغنام العواسي والنعيمي.

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الخلاصة

أجريت التجربة في محطة خيرات الاتحاد الواقعة في قضاء الشوملي بمحافظة بابل للفترة من 1 كانون الثاني 2024 ولغاية 20 حزيران 2024 وذلك لتقييم تأثير بعض المعاملات الهرمونية على معايير الكفاءة التناسلية للنعاج العواسية والنعيمي لزيادة الانتاج المحلي وتحقيق 2-3 مواليد في السنة من النعجة الواحدة ، وتم اختيار أربعة وعشرين رأساً من الأغنام العواسية وأربعة وعشرين رأساً من الأغنام النعيمي وقسمت إلى ثمانية مجاميع بواقع ستة أغنام في كل مجموعة (أربع مجاميع من الأغنام العواسية وأربع مجاميع من الأغنام النعيمي). أظهرت النتائج تأثيراً في الوقت على ظهور علامات الشبق في النعاج العواسية ($P \leq 0.05$) ووضعت الاسفنجات المهبلية لمدة 9 أيام وبعد الإزالة، تم حقن 125 ميكروجرام من هرمون البروستاغلاندين و330 وحدة دولية من هرمون مصل الفرس الحامل، تم تسجيل أفضل العلامات بعد 48 ساعة من المعاملة (50%) وتم ملاحظة حوالي 33.33% من علامات الشبق بعد 72 ساعة ولم يتم ملاحظة أي علامات شبق بعد 96 ساعة من المعاملة. وضعت الاسفنجات المهبلية لمدة 9 أيام وبعد الإزالة، تم حقن 125 ميكروجرام من هرمون البروستاغلاندين و1 مل من هرمون الكونادوتروبين (المعاملة 2) واثرت بشكل كبير ($P \leq 0.01$) على ظهور علامات الشبق. كان هناك فرق معنوي بين النعاج المعالجة بالمعاملة الرابعة والتي استخدم فيها أداة CIDR لمدة 12 يوم وبعد ازالته تم حقن 125 ميكروجرام من هرمون البروستاغلاندين و1 مل من هرمون الكونادوتروبين وسجل أفضل معدل لعلامات الشبق بعد 48 ساعة من المعاملة بينما كانت المعدلات 16.67 و 16.67 و 0.00% بعد 24 و 72 و 96 ساعة من المعاملة على التوالي. أظهرت النتائج وجود فروق معنوية في معدل ظهور علامات الشبق لسلالة أغنام النعيمي التي عوملت بمعاملات تناسلية مختلفة في أوقات مختلفة، اذ اختلفت النعاج التي عوملت بالاسفنج لمدة 9 أيام وبعد إزالة 125 ميكروجرام من هرمون البروستاغلاندين و1 مل من هرمون الكونادوتروبين بشكل معنوي ($P \leq 0.05$) في وقت ظهور علامات الشبق، اذ لوحظ أعلى معدل عند 48 ساعة بعد العلاج (50.0%) بينما سجل أدنى معدل للعلامات عند 96 ساعة بعد العلاج (0.0%). كما اختلف معدل ظهور علامات الشبق بشكل معنوي ($P \leq 0.05$) بين النعاج التي عوملت ببروتوكولات مختلفة بعد 48 ساعة من العلاج، اذ لوحظ أعلى معدل في النعاج بالمعاملة الثانية 50% بينما كانت المعدلات 33.33 و 33.33 و 0.00% في المعاملات الأولى والثالثة والرابعة على التوالي. وكانت نسبة الحمل في النعاج العواسية والنعيمي 100% و 91% على التوالي.

الكلمات المفتاحية: اغنام عواسي ونعيمي، علامات الشبق، الهرمونات، الاسفنجات.

INTRODUCTION

Sheep are important animals and contribute significantly to the agricultural economy and they play a crucial role in Iraq and the Arab world, as the production of sheep meat, milk, and wool constitutes a large percentage of animal production in the region (Kadhim *et al.*,2014; Asjibode *et al.*,2017; Rahman *et al.*,2021). Synchronization of estrus in sheep is an important and widely used technique to overcome the challenges related to seasonal breeding patterns (Ozbilek *et al.*,2022). In sheep breeding, natural estrus still poses a challenge for many breeders. Therefore, various programs have been implemented to improve the utilization of ewes by synchronizing estrus, which effectively enhances reproduction (Mohamad & Hmod,2013 ; Abdulkareem *et al.*,2014 ; Al-Jubouri & Hobi,2016) On a large scale in breeding farms, it works to shorten the gestation period and achieve two to three births per



year, Estrus synchronization is an excellent management tool that has been used with great effectiveness in conjunction with artificial insemination and natural insemination. (Al-Temimi *et al.*,2010), thereby reducing the economic costs of breeding and increasing the number of lambs and obtaining more economic returns (Al-Khazraji *et al.*,2000; Almaamory & Al-Anbari,2023; Rosasco *et al.*,2019). There are many hormonal programs to synchronize estrus in sheep, and the most important and widespread are vaginal sponges, prostaglandins, and their analogues through intramuscular injection (Fadhil *et al.*,2017; Habeeb & Kutzler ,2021).

Many past studies reported that the signs of estrus are affected by many factors such as using the CIDR device for five days and the third group of sheep were injected with PGF2 α hormone on the fifth day after removing the CIDR device. The fourth group of sheep were injected with GnRH and PGF2 α hormones on the fifth day. It was found that the estrus rate was higher in the fourth group and there was no difference in pregnancy rate among the four groups (Jackson *et al.*,2014). The CIDR tool for different periods on the rate of estrus appearance and pregnancy rate (Swelum *et al.*,2018). In another study, the ewes were divided into three groups. The first group was control, the second group was treated with CIDR for 12 days, and the third group was injected with 10 mg of PGF2 α hormone after two and a half days from the start of the breeding season. The second group of ewes had a higher estrus rate and pregnancy rate (Silva *et al.*,2021).

The major aim of this study is to investigate the effect of many reproductive protocols such as vaginal sponges, CIDR and concentrations of GnRH, ECG and PGF2- alpha on the appearance of estrus signs in different times of Awassi and Naimi sheep breeds and exploiting the results to improve the reproductive performance of domestic sheep and enhance the reproductive efficiency in local flocks.

MATERIALS AND METHODS

Animals and management:

The experiment was conducted at the Khairat Al-Ittihad station located in the Al-Shumali district of Babil Governorate from January 1, 2024, to June 20, 2024, to assess the impact of certain hormonal treatments on the reproductive efficiency parameters of Awassi and Naimi ewes. Twenty-four Awassi sheep and twenty-four Naimi sheep were selected and divided into eight groups, with six sheep in each group (four groups of Awassi sheep and four groups of Naimi sheep).

The housing system at the station is semi-intensive, as the station contains rooms equipped with all modern rearing tools and good building specifications. The sheep are fed concentrated feed and roughage three times a day, which are at twelve noon, eight in the morning, and four in the afternoon, with the availability of salt blocks and clean water. The raw materials that make up the concentrated feed are also available.

All the selected sheep for the experiment have been confirmed to be in good health and free from reproductive diseases and defects. They are being supervised by a veterinarian and have been vaccinated with the Entero-Turkish vaccine, which works against the Costeridium bacteria, and the vaccine for foot-and-mouth disease. The origin of the vaccine for sheep pox is



Canadian, and treatments for uterine inflammation, namely Pinsllin of Turkish origin and Diperon of Saudi origin, were administered.

Reproductive protocols (treatments):

The 1st treatment was using vaginal sponges containing 60 mg of progesterone were used. The sheep were immobilized and the mechanism was raised upwards. The vaginal area was cleaned with disinfectants and the vaginal sponges were inserted into the vagina using a sponge inserter, which is a tube in which the sponge is placed while the sponge thread remains outside. The tube was sterilized. The 2nd treatment was the same steps as the first method, except inject 1 ml of GnRH hormone instead of PMSG hormone.

The 3rd treatment was the CIDR tool used, which contains 0.3 grams of progesterone. The 4th treatment was the same steps as the third method, except inject 1 ml of GnRH hormone instead of PMSG hormone.

Signs of estrus were observed on the ewes in the eight groups at the end of each transaction, and six rams were released with the ewes for five days. Any ewe showing signs of estrus was presented to the rams and was inseminated twice by the ram. A color was applied to each inseminated ewe, and she was isolated from the flock.

Statistical analysis:

Data were analyzed by using statistical analysis program (SAS,2012). As the significant differences between the percentages were compared using the Chi-Square (χ^2) test, especially for reproductive efficiency indicators (observation numbers and expected numbers) at the probability levels of 0.05 and 0.01.

RESULTS AND DISCUSSION

Results showed a significant effect of time (period) of protocols or sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected on estrous signs appearance in Awassi ewes ($P \leq 0.05$), the best signs were recorded after 48 hours from treatment (50%) and about 33.33% of estrus signs were noticed after 72 hours and 16.67 after 24 hours no estrus signs were seen 96 hours after treatment (Table-1).

Results showed that the sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected (treatment 2) effected significantly ($P \leq 0.01$) on estrous signs appearance. The highest signs rate was noticed after 48 hours from treatment namely, 66.67% while the rate after 72 hours was 33.33% and no estrus signs were seen after 96 hours after treatment.

The rate of estrus signs appearance did not differ significantly among the ewes that treated with the 3rd treatment which including CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected. Results showed a significant difference among the ewes that treated with 4th treatment which including CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected, the best estrus signs rate was recorded after 48 hours from treatment while the rates were 16.67, 16.67 and 0.00% after 24, 72 and 96 hours of treatment respectively.

**Table (1):** Comparison among protocols in estrous signs for Awassi ewes.

treatments	Time (hr)				Significance
	24	48	72	96	
	No.(%)	No.(%)	No.(%)	No.(%)	
T1	1 (16.67%)	3(50.0%)	2(33.33%)	0) 0.00%(	*
T2	0(0.00%)	4 (66.67%)	2(33.33%)	0) 0.00%(	**
T3	2(33.33%)	1 (16.67%)	2 (33.33%)	1) 16.67%(	N.S.
T4	1(16.67%)	4 (66.67%)	1(16.67%)	0(0.00%)	**
Significance	*	**	N.S.	N.S.	

no significant NS ,($P \leq 0.01$) **, ($P \leq 0.05$) *

Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected. T2: Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected. T3: CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected. T4: CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected.

The treatment differed significantly ($P \leq 0.05$) in the rate of estrus signs appearance after 24 hours of injection, the highest rate was recorded in ewes of 3rd treatment (33.33%) while the lowest rate was recorded in ewes of 2nd treatment (0.00%). Estrus signs appearance rate differed significantly ($P \leq 0.01$) among ewes that treated with different protocols after 48 hours of treatment, the highest rate was noticed in ewes with 2nd and 4th treatments namely, 66.67% while the rates was 50 and 16.67 % in ewes that treated with 1st and 3rd treatments respectively. No significant differences among ewes that treated with the different protocols in estrus signs appearance at 72 and 96 hours after treatments.

Results showed a significant difference in the appearance rate of estrus signs of Naimi sheep breed that treated with different reproductive treatments in different times (Table-2). The ewes that treated with sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected differed significantly ($P \leq 0.05$) in the time of estrous signs appearance, the highest rate was noticed at 48 hours after treatment (50.0%) while the lowest rate of signs was recorded at 96 hours after treatment (0.0%).

The ewes that treated with CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected differed significantly ($P \leq 0.05$) in the time of estrous signs appearance, the highest rate was noticed at 24 and 96 hours after treatment (33.33%) while the lowest rate of signs was recorded at 48 hours after treatment (0.0%).

The time of estrus signs appearance did not differ significantly among ewes that treated with 1st treatment (Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected) and also not differed significantly in ewes that treated with the 3rd treatment (CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected).

Estrus signs appearance rate differed significantly ($P \leq 0.05$) among ewes that treated with different protocols after 48 hours of treatment, the highest rate was noticed in ewes with 2nd treatment namely, 50.00% while the rates was 33.33, 33.33 and 0.00 % in ewes that treated



with 1st, 3rd and 4th treatments respectively. No significant differences among ewes that treated with the different protocols in estrus signs appearance at 24, 72 and 96 hours after treatments.

Table (2): Comparison among protocols in estrous signs for Naimi ewes.

treatments	Time (hr)				Significance
	24	48	72	96	
	No.(%)	No.(%)	No.(%)	No.(%)	
T1	1(16.67%)	2(33.3%)	2(33.33%)	1(16.67%)	N. S
T2	1(16.67%)	3(50.00%)	2(33.33%)	0(0.00%)	*
T3	1(16.67%)	2(33.33%)	1(16.67%)	1(16.67%)	N.S.
T4	2(33.33%)	0(0.00%)	1(16.67%)	2(33.33%)	*
Significance	N. S	*	N.S.	N.S.	
no significant NS (P≤0.05) *					

Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected. T2: Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected. T3: CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected. T4: CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected.

The current results are similar with many past results which referred to a significant difference among ewes in the time of estrus signs appearance (Aqwaan ,2023; Nasrin *et al.*,2018) reported for comparison between the effect of using CIDR for a short time and a long time, the sheep were divided into two groups. The first group had CIDR inserted for 11 days, and the second group had CIDR inserted for 14 days. When removed, all sheep in both groups were injected with 300 international units of eCG hormone And blood samples were collected to measure the levels of progesterone and estradiol hormones. The levels of progesterone hormone remained the same after the removal of the tool, but after 48 hours, the estradiol hormone was much higher. The response to appetite was similar in both the first and second groups. The results also supported by (Martinez-Ros *et al.*,2019; Miguel-Cruz *et al.*,2019 ; Kuru *et al.*,2022).

In another study comparing different hormonal programs, the ewes were divided into three groups and a CIDR device was inserted for five days. Ewes in the first group were injected with 400 international units of eCG hormone and 5 milligrams of PGF2 α hormone on the fifth day. Ewes in the second group were injected with 50 micrograms of GnRH hormone and 5 milligrams of PGF2 α hormone on the fifth day as well. Ewes in the third group were injected with 50 micrograms of GnRH hormone when placing a CIDR device and removing it on the fifth day, 5 milligrams of PGF2 α hormone and 50 micrograms of GnRH hormone were injected. The rate of estrus appearance was similar in all groups, but the first group showed signs of estrus early and the fertility rate was 76.5%, 64.7%, and 81.3% respectively (Martinez *et al.*,2015).

Pinna *et al.* (2012); Vasconcelos *et al.* (2016); Dias *et al.* (2015) reported a similar result and noticed that Using CIDR reduces the incidence of infections compared to vaginal sponges because they do not adhere to the vagina. The design and structure of the CIDR device allow for better drainage of vaginal secretions. In fact, around 15% of sheep undergoing estrus



synchronization using CIDR show vaginal secretions, which are rare. Others found a higher pregnancy rate using the previously used CIDR tool over the long term (11 days) (**Martinez et al.,2015; Pinna et al., 2012; Vasconcelos et al. 2016**). Therefore, it is possible that when reusing the IDRC tool, the optimal level of progesterone concentration can be achieved to increase follicle growth. To maintain the prevailing coherence [26], and to determine which one has a greater impact on reproductive performance variables and serum progesterone concentration in the blood of ewes, a comparison was made between the new and used tool, and no difference was observed in the rate of estrus appearance, pregnancy rate, fertility rate, and type of birth. The rate of estrus appearance in ewes ranged from 93-100%, with an average pregnancy rate of 80.8% and an average fertility rate of 80.2% (**Dias et al.,2015 ;Alvarez et al.,2013; Al-Marzani & Barwary,2022**).

CONCLUSION

In conclusion, the current results proved that the estrus signs appearance can altered by using some of reproductive protocols therefore, using of efficient protocols in the suitable time is very useful to enhance the reproductive efficiency in the local sheep breed which suffer from weakness and unclearness of estrus signs.

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