

Impact of Various Protocols of Estrus Synchronization in Some of Reproductive Hormones for Two Sheep Breeds in Iraq.

Ali Abd Almohsin Al-Talebi¹, Husam Jasim Hussein Banana²

^{1,2}Department of Animal production, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.

Email: ali.abd2201p@coagri.uobaghdad.edu.iq, husam.banana@coagri.uobaghdad.edu.iq

Received: 12.11.2024

Revised: 17.12.2024

Accepted: 11.01.2025

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 effect of hormonal treatments on estrogen and progesterone hormones concentrations of Awassi and Naimi ewes. Results showed a significant effect ($P < 0.01$) of treatment (before and after) on estrogen concentration for both 1st and 2nd treatments in Awassi sheep breed. The estrogen concentration after treatment was 39.74 pg/ml compared with its concentration before treatment which was 50.96 pg/ml in 1st treatment. The estrogen level in 2nd treatment differed significantly before and after treatment (53.08 and 42.94) pg/ml respectively. Results indicated that the estrogen levels differed significantly before and after treatment in all treatments for Naimi sheep breed. In case of 1st, 2nd and 3rd treatment, the estrogen hormone level differed significantly ($P < 0.05$) before and after treatments. The hormones levels were 43.25, 41.66 and 42.34 pg/ml after treatment respectively compared with 51.73, 50.76 and 50.44 pg/ml before treatment respectively. The 4th treatment effect significantly ($P < 0.01$) on estrogen hormone level, the level was 40.83 pg/ml after treatment compared with 50.89 pg/ml before treatment. Awassi sheep group that treated with 4th treatment showed a significant difference in progesterone hormone level namely, 1.38 and 5.36 ng/ml respectively. The difference between progesterone level before and after treatment in Naimi was highly significant ($P < 0.01$) for all treatments.

Keywords: progesterone, Reproductive protocols, Sheep breed.

1. INTRODUCTION

Awassi sheep can be classified as the most famous and important sheep breed in middle east counties as well as in other regions around the world. The breed characterized as multipurpose but many of countries improved it to be a good dairy sheep breed in addition to its mutton from lambs fattening (1,2). Sheep are important animals and contribute significantly to the agricultural economy and they play a crucial role in Iraq as the production of sheep meat, milk, and wool constitutes a large percentage of animal production in the region (3,4).

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 (5,6,7).

On a large scale in breeding farms, it works to shorten the gestation period and achieve two to three births per year, thereby reducing the economic costs of breeding (8) and increasing the number of lambs and obtaining more economic returns (9,10,11).

Vaginal sponges with progestin activity can be divided into medium and long-acting (9-16 days) And short-term (5-7 days) studies have shown that long-term use (11 days) significantly increases the pregnancy rate in ewes compared to short-term use (7 days) (12,13). Another important method for synchronizing estrus is the use of CIDR technology for 14 days, which also contains progesterone hormone (14).

The prostaglandin and its derivatives are effective factors for the luteolysis, and they have been widely used to synchronize estrus in ewes (15). The combined use of progesterone and prostaglandin has a good effect on estrus synchronization in ewes. Prostaglandin is usually administered before the sponge insertion (16,17).

The aim of this study is to determine the effect of four different programs for some of reproductive hormones (estrogen and progesterone) in local ewes and select the most practical program for increasing of reproductive efficiency in local ewes by comparing four different programs and using of the results as guidelines to improve the local domestic sheep.

2. 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 Painslin 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.

The sexual hormones such as estrogen and progesterone were measured by special kit and then it's considered as marker for reproductive efficiency to detect the effect of the protocols on the hormones levels which reflect on reproductive performance.

Statistical analysis: Data were analyzed by using statistical analysis program (18) according to completely randomized design (CRD) and tow way ANOVA procedure was flowed to determine the effect of treatment on hormones concentrations (vertically) and the effect of each treatment (before and after) on hormones concentrations (horizontally). The significant differences were determined by Duncan multiple range test Duncan (19) under the linear model:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

Where: μ : the overall mean

T_i : effect of treatments on hormones concentrations.

e_{ijk} : is a random error.

3.RESULTS AND DISCUSSION

Statistical analyzing shows a significant effect ($P < 0.01$) of treatment (before and after) on estrogen concentration for both 1st and 2nd treatments in Awassi sheep breed (Table-1), in case of 1st treatment, the estrogen concentration after treatment was 39.74 pg/ml compared with it concentration before treatment which was 50.96 pg/ml. The estrogen level in 2nd treatment differed significantly before and after treatment (53.08 and 42.94) pg/ml respectively. No significant differences in estrogen levels before or after treatment in 3rd and 4th treatments for treated Awassi breed.

The estrogen level did not differ significantly among treated Awassi sheep with all treatments after treatment as well as did not differ significantly among sheep after treatments.

Results indicated that the estrogen levels differed significantly before and after treatment in all treatments for Naimi sheep breed. In case of 1st, 2nd and 3rd treatment, the estrogen hormone level differed significantly ($P < 0.05$) before and after treatments. The hormones levels were 43.25, 41.66 and 42.34 pg/ml after treatment respectively compared with 51.73, 50.76 and 50.44 pg/ml before treatment respectively.

4th treatment effect significantly ($P < 0.01$) on estrogen hormone level, the level was 40.83 pg/ml after treatment compared with 50.89 pg/ml before treatment.

The estrogen hormone level did not differ significantly among treatment before or after treatments, due to the values of before treatments, the hormones level was ranged for 50.44 pg/ml in 3rd treatment to 51.73 pg/ml in 1st treatment while the range of hormone level after treatment was 40.83 pg/ml in 4th treatment to 43.25 pg/ml in 1st treatment.

Table 1: Effect of treatments on estrogen hormone in sheep blood

Breed	treatment	Mean and± sd (PG/ML)		Significance
		After treatment	Before treatment	
Awassi	T1	2.11±39.74	2.48±50.96	**
	T2	2.42±42.94	2.23±53.08	**
	T3	3.85±45.99	2.43±51.23	N.S.
	T4	3.42±42.45	3.72±49.15	N.S.
		N.S.	N.S.	Significance
Naimi	T1	2.27±43.25	2.59±51.73	*
	T2	2.71±41.66	2.89±50.76	*
	T3	2.57±42.34	2.75±50.44	*
	T4	2.27±40.83	2.46±50.89	**
		N.S.	N.S.	

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.

Results represented in Table -2 showed a high significant effect of treatment type on progesterone level in Awassi sheep breed. According to the 1st treatment, the progesterone level was 0.89 ng/ml before treatment while it became 4.09 ng/ml after treatment ($P<0.01$). In sheep that treated with 2nd treatment (Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected), the progesterone level before and after treatment was 0.885 and 4.10 ng/ml respectively while in sheep under 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), the hormone level before and after treatment was 1.23 and 5.53 ng/ml respectively. Awassi sheep group that treated with 4th treatment (CIDR device for 12 days and after removal, 125 micrograms of PGF2 α hormone and 1 ml of GnRH hormone were injected) showed a significant difference in progesterone hormone level namely, 1.38 and 5.36 ng/ml respectively.

Results of Naimi sheep breed were coming in the same direction of Awassi group. The difference between progesterone level before and after treatment was highly significant ($P<0.01$) for all treatments. In 1st treatment (Sponges for 9 days and after removal, 125 micrograms of PGF2 α hormone and 330 international units of eCG hormone were injected), the hormones values were 1.178 and 4.32 ng/ml before and after treatment respectively. In 2nd treatment the hormones levels were 1.166 and 4.53 ng/ml respectively. In 3rd treatment the hormones levels were 1.138 and 4.43 ng/ml before and after treatment respectively. In 4th treatment the hormones levels were 1.090 and 4.88 pg/ml before and after treatment respectively.

The progesterone hormone level did not differ significantly among treatment before or after treatments, due to the values of before treatments, the hormones level was ranged for 0.885 ng/ml in 2nd treatment to 1.38 ng/ml in 4th treatment while the range of hormone level after treatment was 4.09 ng/ml in 1st treatment to 5.53 ng/ml in 3rd treatment.

Table 2: Effect of treatments on progesterone hormone in sheep blood

Breed	treatment	Mean and± sd (NG/ML)		Significance
		After treatment	Before treatment	
Awassi	T1	0.037±4.09	0.08±0.890	**
	T2	0.048±4.10	0.13±0.885	**
	T3	0.040±5.53	0.024±1.23	**
	T4	0.021±5.36	0.031±1.38	**
		*	N.S.	Significance
Naimi	T1	0.037±4.32	0.017±1.178	**
	T2	0.044±4.53	0.024±1.166	**
	T3	0.051±4.43	0.021±1.138	**
	T4	0.027±4.88	0.018±1.090	**
		N.S.	N.S.	

T1: 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 were generally agreed with many past results which referred that the reproduction assisted technologies effect significantly on major sexual hormones levels which reflect positively on reproductive performance in sheep breeds around the world (20,21,22,23). The results of this study also support the results of (24,25) who reported that the reproduction of small ruminants can be controlled by the administration of exogenous hormones including multiple treatments.

The results are similar with results of (26,27) who reported that the small ruminant's reproduction performance is controlled by many technologies such as estrus synchronization and lead to modify the physiological chain of events involved in the estrus cycle and hormones levels such as estrogen and progesterone. The results are agreed with those of (28,29) who reported that the estrogen treatment increase the insulin sensitivity and effect on reproductive performance.

Many past studies reported that the Various estrus synchronization technologies that developed in ewes, using natural or synthetic progesterone in combination with gonadotropins and prostaglandins effect significantly on both estrogen or progesterone level in blood which led to allow the constancy of the results irrespective of the season (30,31,32).

4.CONCLUSION

the hormonal treatments of sheep local breeds which aim to estrous timing might possibly lead to increase the levels of reproductive hormone such as estrogen or progesterone in synchronization protocols.

REFERENCE

1. Amin, A.Y. and K. J. Peters. 2006. Awassi Sheep Production and the Development of breeding Program Options in Syria. Tropentag, October 11-13, 2006, Bonn "Prosperity and Poverty in a Globalised World. Challenges for Agricultural Research".
2. Fadhil Abbas Al-Zubaidi, S., Bakir Abdul-Hussain, Z., & Fadhil Abdul-Hussain, I. (2017). Vaginal microflora in ewes after estrus synchronization with intravaginal sponges. In The Iraqi Journal of Veterinary Medicine.41(2):67-71.
3. Rahman H. H. Al Qasimi 1 , Azhar. A. Jaffar2 & Allawi L. D. ALKhauzai. Relationship GDF9B (BMP-15) Gene Polymorphism with litter size in Iraqi Awassi Sheep. Euphrates Journal of Agricultural Science2021, Volume 13, Issue 4, Pages 187-194.
4. Al-Ani, A. ; M.A. Ishak and M.A. Al-Ekpi. 2010. Detection of uterine involution using ultrasonography in Turkish Awassi ewes. Iraqi Journal of Agricultural Sciences2010, Volume 41, Issue 5, Pages 117-124.
5. Mohamad A. Ishaq, Hmod M. Ajeel.2013.Reproductive performance characteristics of local and Turkish Awassi sheep in semi-intensive system. Iraqi Journal of Agricultural Sciences.2013, Volume 44, Issue 5, Pages 615-623.
6. Abdulkareem, T. A.; S. M. Eidan ;L. A. Al-Maliki and F. K. Al-Saidy.2014. Reproductive performance of Iraqi Awassi ewes owned by sheep owners and extension farms in response to flushing and estrus system synchronization regimes. The Iraqi Journal of Agricultural Sciences – 45(3) (Special Issue): 328-334.
7. Al-Jubouri, A. D.; A. A. Hobi. 2016. Field evaluation of estrus synchronization and pregnancy detection using ultrasonography to the local Awassi ewes for sheep owners in Kanaan district – Diyala province. The Iraqi Journal of Agricultural Sciences – 47(4):1078-1088.
8. Petovic P.M., Ruzic D., Maksimvic N., Memisi N. (2009): Effect of environmental and paragenetic factors on birth mass variability of Mis sheep population. Biotechnology in Animal Husbandry, 25, 213–219.
9. Al-Khazraji, A. A., S. M. Abu- Tabeigh, T. A. Abdulkareem and U. A. Mahdi. 2000. Reproductive responses of Awassi ewes to different level of PMSG administration. The Iraqi Journal of Agricultural Sciences .31: 681-687.
10. Turk G, Gur S, Sonmez M, Bozkurt T, Aksu EH, Aksoy H. 2008. Effect of exogenous GnRH at the time of artificial insemination on reproductive performance of Awassi ewes synchronized with pro-gestagen-PMSG-PGF2 α combination. Reproduction in Domestic Animals. 43:308-313
11. Rosasco, S.L., Beard, J.K., Hallford, D.M., and Summers, A.F., 2019.Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability. Animal Reproduction Science,210, 106191.
12. Garoussi MT, Mavadati O, Bahonar M, Ragh MJ. 2020. The effect of medroxyprogesterone acetate with or without eCG on conception rate of fat-tail ewes in out of breeding season. Trop Anim Health Prod. 52:1617–22.
13. Habeeb, H. M. H., & Kutzler, M. A. 2021. Estrus synchronization in the sheep and goat. Veterinary Clinics: Food Animal Practice, 37(1), 125-137.

14. Jackson, C. G., Neville, T. L., Mercadante, V. R. G., Waters, K. M., Lamb, G. C., Dahlen, C. R., & Redden, R. R. (2014). Efficacy of various five-day estrous synchronization protocols in sheep. *Small Ruminant Research*, 120(1), 100–107. <https://doi.org/10.1016/j.smallrumres.2014.04.004>
15. Swelum, A.A.-A., Saadeldin, I.M., Moumen, A.F., Ali, M.A., and Alowaimier, A.N., 2018. Efficacy of controlled internal drug release (CIDR) treatment durations on the reproductive performance, hormone profiles, and economic profit of Awassiewes. *Small Ruminant Research*, 166, 47-52.
16. Rosasco, S.L., Beard, J.K., Hallford, D.M., and Summers, A.F., 2019. Evaluation of estrous synchronization protocols on ewe reproductive efficiency and profitability. *Animal Reproduction Science*, 210, 106191.
17. Aryaa, D.; R. Goswami and M. Sharma. Estrous synchronization in cattle, sheep and goat. <https://doi.org/10.31893/multirev.2023001>.
18. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9. 1th ed. SAS. Inst. Inc. Cary. N.C. USA.
19. Duncan, D.B. 1955. Multiple Range and Multiple F- Tests. *Biometrics*, 11 :1- 41.
20. Martinez-Ros P, Rios-Abellan A, Gonzalez-Bulnes A (2019) Influence of progesterone-treatment length and eCG administration on appearance of estrous behavior, ovulatory success and fertility in sheep. *Animals* 9:9.
21. Miguel-Cruz EE, Mejía-Villanueva O, Zarco L (2019) Induction of fertile estrous without the use of steroid hormones in seasonally anestrous Suffolk ewes. *Asian-Australas J Anim Sci* 23:1673-1685.
22. AL-Jaryan, I.L., AL-Thuwaini, T.M. & Al-Jebory, H.H. Novel variants associated with adiponectin-related traits in Awassi ewes. *Beni-Suef Univ J Basic Appl Sci* 11, 148 (2022). <https://doi.org/10.1186/s43088-022-00328-7>
23. Kocakaya A, Özbeyaz C .2019. The Effects of Progesterone and Estrogen Hormone Levels on Some Reproductive Characteristics of The Akkaraman Sheep. *KSU J. Agric Nat.* 22(Suppl 2): 424-430.
24. Pinna, A., Brandão, F., Cavalcanti, A., Borges, A., Souza, J., & Fonseca, J. (2012). Reproductive parameters of Santa Inês ewes submitted to short-term treatment with re-used progesterone devices. *Arquivo Brasileiro De Medicina Veterinária E Zootecnia*, 64, 333–340. <https://doi.org/10.1590/S0102-09352012000200012>.
25. Vasconcelos, C. O. P.; Brandão, F. Z.; Martins, G.; Penna, B.; Souza-Fabjan, J. M. G. and Lilenbaum, W. 2016. Qualitative and quantitative analysis of bacteria from vaginitis associated with intravaginal implants in ewes following estrus synchronization. *Ciência Rural* 46:632-636. <https://doi.org/10.1590/0103-8478cr20150365>.
26. Dias, L. M. K.; Paes de Barros, M. B.; Viau, P.; Sales, J. N. S.; Valentim, R.; Santos, F. F.; Cunha Jr., M. C.; Marino, C. T. and Oliveira, C. A. 2015. Effect of a new device for sustained progesterone release on the progesterone concentration, ovarian follicular diameter, time of ovulation and pregnancy rate of ewes. *Animal Reproduction Science* 155:56-63. <https://doi.org/10.1016/j.j>
27. Alvarez, L.; Gamboa, D.; Zarco, L. and Ungerfeld, R. 2013. Response to the buck effect in goats primed with CIDRs, previously used CIDRs, or previously used autoclaved CIDRs during the non-breeding season. *Livestock Science* 155:459-462. <https://doi.org/10.1016/j.livsci.2013.05.010>
28. Garcia-Arevalo M, Lorza-Gil E, Leite N, Brunetto S, Boschero AC, Carneiro EM. The 17-Beta-Estradiol Improves Insulin Sensitivity in a Rapid Estrogen Receptor Alpha-Dependent Manner in an Animal Model of Malnourishment. *J Endocrinol Metab* (2019) 9:133–46. doi: 10.14740/jem612 [DOI] [Google Scholar]
29. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The Role of Estrogens in Control of Energy Balance and Glucose Homeostasis. *Endocr Rev* (2013) 34:309–38. doi: 10.1210/er.2012-1055 [DOI] [PMC free article] [PubMed] [Google Scholar]
30. AL-Jaryan, I. L., AL-Thuwaini, T. M., & AL-Jebory, H. H. (2023). Heat shock protein 70 and its role in alleviating heat stress and improving livestock performance. *Reviews in Agricultural Science*, 11, 234-242.
31. Sharma and Sudhir Kumar. 2023.. Estrus synchronization in sheep using progesterone sponge Anil Kumar Pandey, Utsav. *The Pharma Innovation Journal* 2023; SP-12(6): 500-502.
32. Nakafeero A, Hassen A, Lehloenya K (2020) Investigation of ram effect and eCG usage in progesterone based oestrous synchronization protocols on fertility of ewes following fixed time artificial insemination. *Small Ruminant Research* 183:106034.