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Effect of utilizing different synchronizing protocols on reproductive efficiency of local Iraqi sheep

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Abstract In sheep farming, one of the most crucial economic characteristics is increasing the number of livestock wondering for female newborns. This study aimed to investigate the middle-aged Iraqi sheep breed and determine its correlation with litter size by employing three distinct synchronization methods on the reproductive capabilities of Iraqi ewes grown in mid-southern Iraq. In a randomized block experimental design, eighty local ewes, aged three to four years old, were divided into four groups for the purpose of estrus synchronization: group 1 (n = 20 control animals), group 2 (n = 20 Medroxy Progesterone Acetate (MAP) sponges only), group 3 (n = 20 MAP sponges and GnRH), and group 4 (n = 20 MAP sponge and equine chorionic gonadotropin (eCG)). First, an ANOVA was applied to the data on estrus response, insemination, pregnancy, and prolificacy, followed by a chi square test. At ($p \leq 0.05$), the results were deemed significant. Over 80% of the ewes tested were response positively for estrus according to the group 2, which showed an intriguing success estrus rate. More than 75% of the ewes examined in Group 3 procedure demonstrated estrus. When the procedures were compared, group 3 outcomes for positive estrus, pregnancy rates, and litter size were superior. Therefore, it can be said that all tested protocols yielded outcomes that were good in terms of prolificacy, or the number of lambs produced per ewe, and estrus manifestation in compared to control group. While other treatments with the presence of the ram effect may improve the lambing rate and raise litter size, the MAP only protocol demonstrated higher findings for positive indicator of female pregnancy 66.6%.

Keywords: eCG, ewes, GnRH, litter size, pregnancy, sex ratio, sponges

Introduction Livestock production depends on reproduction to ensure the survival of the economic production system. Selling lambs is said to generate at least 60% of farm profits in the area; the commodity's value is determined by factors such as the lambing interval rate, and overall reproductive effectiveness (1). The length of the photoperiod, latitude/longitude, season variations, and other factors influence the reproductive activity of most farm animal breeds (2). Due to this, a number of techniques have been employed to regulate ovarian activity with the goal of increasing small ruminant fertility (3) and avoiding anestrus, the most prevalent reproductive complaint in ewes, which results in decreased fecundity and significant financial losses for the farmers (4).

Inducing or synchronizing estrus is an stimulating method for raising ewes pregnancy rates. The efficiency of extensive production has increased appreciatively to modern ewe husbandry, which has also managed the reproductive process for intense production. In general, the synchronization of estrus in ewes is centered on controlling the estrus cycle, namely the luteal or follicular phase (5). For this reason, hormone therapy to regulate ovulation and reproduction presents a captivating substitute for effective in vitro fertilization and boosting the quantity of fecund females (2,6). Estrus synchronization is the main goal of exogenous hormone applications for improved reproductive success in domestic ewes (7).

According to Martemucci et al. (2010) (8), the ovarian follicular dynamics have been described as wave-like, with follicular dominance affecting the effectiveness of synchronization during the ewe's estrous cycle and anestrus. Due to its longer length and increased responsiveness to manipulation, the luteal phase offers ewes the most chance for control. There are several methods for estrus synchronization by either shorten the luteal phase by regressing preexisting corpora lutea by PGF2α or to lengthen it by providing exogenous progesterone (9).

The tendency of many breeds of small ruminants to bear and nurture numerous offspring presents a second opportunity. This tendency may be managed by modifying dose levels and manipulating feeding as part of an estrus synchronization program (10). Progesterone-based treatments are often linked to prostaglandin and equine chorionic gonadotropin (eCG) (11). Longer treatment durations tend to prolong the progestogen maintenance period since they might impair follicular growth and reduce fertility (12).

Numerous techniques have been devised to regulate the reproductive behavior of farm animals. The main objective of these treatments is to increase the effectiveness of reproduction by utilizing various hormones (15,16). Ewes are seasonal polyestrous in nature, and they lack sexual activity during the non-breeding season; therefore, hormone therapy is required to induce estrus at this time (13, 17,18). Progesterone and its derivatives are helpful in synchronizing and inducing estrus in sheep (17). When the progesterone-impregnated sponge is inserted intravaginally for ten to fourteen days, estrus is noticed thirty to thirty-seven hours after the sponge is removed (18). Progesterone treatment should be combined with equine chorionic gonadotropin (eCG) (13,19), a hormone that simultaneously functions as both luteinizing hormone and follicle stimulating hormone. When gonadotropin levels are high enough to trigger preovulatory events, progesterone therapy is beneficial; the gonadotropic effect is produced by an eCG injection (20).

Progesterone and/or prostaglandin hormones can be used to either advance or postpone estrus in order to achieve estrus synchronization. Additionally, gonadotropins can be utilized alone or in conjunction with other hormones to control the estrous cycle. For ewes, the MAP, Fluorogestone Acetate progesterone (FGA), and controlled internal drug release (CIDR) devices, all work comparably well to synchronize estrous. Though they are only helpful during the breeding season, prostaglandins are often utilized to synchronize the estrous cycle in sheep. The gonadotropin enhancers (eCG and GnRH) and the ram effect are commonly used to boost progestagen-based treatments' efficacy. Similar to how it's normal practice for sheep to synchronize estrous either during or outside of the breeding season, owners of dairy herds are interested in breeding their animals outside of the breeding season. Estrus synchronization is a financially advantageous practice because it reduces the time between calving and breeding, fixes the breeding time, improves the survival of newborns in suitable environments or seasons when food is available, manages large-scale farms by establishing chronologically consistent reproductive events, and makes it easier to divide up seasonal labor tasks more effectively. All of these factors improve efficiency in terms of offspring survival rates and financial gains. For many domestic ruminants, a successful estrous synchronization program requires proper nutrition and sound farm management practices; nonetheless, more farmers need to be convinced to employ this tactic (21).

For the purpose of raising sheep, genetic and molecular indicators of litter size are fundamental (22). Reproductive efficiency, mutton, and wool qualities are three of a sheep's economic features (23,24). One method to increase sheep reproductive efficiency is to increase litter size, but according to Janssens et al. (2004) (25), sheep litter size is a quantitative feature with a heritability of no more than 20%.

Material and Methods

Study location

This experiment was conducted on a breeder's farm located at the coordinates 32.2886204, 44.5883852, Babylon Governorate, Al-Qasim City in mid-southern Iraq.

Ethical approval

The current study procedures were approved by the College of Veterinary Medicine, University of Al-Qadisiyah.

Experimental Design

In June–July 2023, Neomi local ewes (number = 80) were selected randomly weighing 25-30 kilograms were divided into four groups. Prior to treatment administration, all ewes were fed on alfalfa (3 kg/ewe) and a 14% crude protein concentrate ration (1 kg/ewe) daily. Ewe estrous synchronization treatment groups were as follows:

1) Untreated control group given injection of 2ml normal physiological saline, (n= 20).
2) 14 days sponge MAP insert only (ESPONJAVET®, Esponja intravaginal impregnada de Medroxiprogesterona acetato (60 mg/esponja), (n = 20).
3) 14 days sponge MAP and PMSG 500 IU (OVISER®, Gonadotropina sérica (PMSG), HIPRA, España) injection at the time sponge MAP withdrawing (n = 20).
4) 14 days sponge MAP and injection GnRH 5.25 microgram at time withdrawing sponge MAP (Gestar, Buserelin Acetate (synthetic gonadotropin releasing hormone) 0.00042g, ARGENTINA intramuscular (n=20) .

Estrus detection and mating after synchronization

Based on age and body condition scorethree evenly split groups of rams were created. After the removal of the sponge, ewes were kept with rams for four days, with a 6:1 female to male ratio. A 4-day period was used to measure heat every 8 hours. The ewe was separated from the flock after it was discovered to be in heat after it mated (17).

Diagnosis of pregnancy

Trans rectal B-mode ultrasonography (PT50, BMV, China) using a 7.5 MHZ Convex array transabdominal transducer was used to diagnose pregnancies between 40-45 days of conception via rectum in standing position (26).

Reproductive parameters

A number of reproductive markers were identified, including sex distribution, litter size, birth type, commencement of estrus, estrus response, conception rate, pregnancy rate, lambing rate, female/male ratio and lamb weight. The formulae of (27) were used to determine reproductive indicators.

Statistical analysis

The statistical program graph pad prism was used to do a statistical analysis on the data collected for the investigation. The ANOVA test was used to compute the group differences. Using the chi-square test, the groups' rates of pregnancy and lambing were statistically assessed.

Results

The findings affecting to estrus synchronization, mean (±SD) estrus response, time to estrus start, and length of estrous phase were assembled in Table 1. All groups showed significant variation in terms of estrus response group 3 (eCG group) showed best rate of estrus response compared with other groups and time of estrus onset. On the other hand, time of estrus duration were significantly ($P \leq 0.05$) more in group 1 (control group) when compared to other treated groups. Pregnancy follow-up and Parturition

The ewes were followed up after mating, and in order to confirm the percentage of pregnant ewes that mated, they were examined using a PT-50 ultrasound device between 40-45 days post mating. The reproductive performance is demonstrated in Table 2. Pregnancy rate and lambing rate were with no significant differences between all groups.

On the other hand multiple birth showed significant difference between group 3 and group 4 ($P \leq 0.05$) and both groups are significantly different from group 1 and 2 ($P \leq 0.05$). While numbers of lambs delivered were remarkably variable in all groups showing higher in groups 3,4,2 and 1 respectively. litter size was higher in group 3 compared with group 4 and both significantly different ($p \leq 0.05$) with groups 1 and 2. While gestation period and birth weight showed no significant difference between all groups.

Discussion

The ewes in the experiment showed a different rate of response to estrus, as group 3 (treated with the eCG 500 IU) at the time of withdrawing the sponge MAP was higher and faster than the group4 (treated with GnRH 5.25 µg) after withdrawing sponge MAP, compared to group2 (sponge MAP) and control group1, these results may be due to the fact of using synchronizing protocols may enhance the response of estrus out of season in ewes by different methods while control group gave slight response to estrus especially all ewes were in presence of rams in the herd which may played a significant effect on control group and gave better response to all groups.

The response rate in group 3 was 80% to estrus with time range 41 hours, while group 4 was 75% response with time range of 56 hours, and group 2 was 60% response with time range of 55 hours, finally group1 (the control group) showed 40% response with time range 68 hours.

The response of GnRH group showed less response rate to estrus than eCG group due to maybe that ewes were not in heat and did not develop any dominant follicle which the GnRH would work on while eCG group had an opportunity to grow follicles faster than other groups in a similar study using CIDR instead of MAP (28). The CIDR-eCG group saw an earlier onset of these events compared to the CIDR-GnRH and MAP groups ($p < 0.05$).

Table 1: Ewes estrus response rate, onset of estrus, and duration of estrus after injection of treatments

Groups	Number of ewes	Rate of estrus response	Time (hrs.) of estrus onset (Mean± SD)	Time (hrs.) of estrus duration (Mean± SD)
Control	20	40% [a]	68.24 ± 1.9^{a}	29.18 ± 0.76^{a}
MAP only	20	60% [b]	55.03 ± 1.1^{b}	24.12 ± 0.96^{b}
MAP+eCG	20	80% [c]	41.37 ± 1.0^{c}	23.96 ± 1.21^{b}
MAP+GnRH	20	75% [bc]	56.09 ± 1.3^{b}	23.54 ± 1.37^{b}

Different letters: Significant difference *p*<0.05.

Table 2: Ewes reproductive parameters of pregnancy rate, lambing rate, multiple birth, number of lambs, litter size, gestation period, birth weight and litter sex and sex ratio of all groups

Parameters	Control group1	Group 2	Group3	Group4
Pregnancy rate	100% (8/8)	100% (12/12)	100% (16/16)	100% (15/15)
Lambing rate	100% (8/8)	100% (12/12)	100% (16/16)	100% (15/15)
Multiple birth	0 % [c]	0 % [c]	43.75% (7/16)[a]	33.33(5/15)[b]
Number of lambs	8	12	30	20
Singles	8	12	3	10
Twins	0	0	12 (24)	5 (10)
Triplets	0	0	1(3)	0
Litter size	1(8/8) [c]	1(12/12)[c]	1.875 (30/16)[a]	1.33(20/15)[b]
Gestation period (days)	149±0.52	150.34±0.87	152.65±1.78	151.07±1.21
Birth weight (kg)	3.25±0.75	3.12±0.25	3.01±0.35	3.17±0.33
Female	5	8	13	12
Male	3	4	17	8
Female/ male ratio	5/3 (1.66)[b]	8/4 (2)[a]	13/17 (0.76)[c]	12/8 (1.5)[b]

Different superscripts (same row): Significantly different (*p*≤0.05)

Since the timing of the LH peak and the intervals between ovulation were identical across treatments, the initiation of estrus behavior was primarily responsible for these changes. Our study showed better results due to good management and environmental effects especially in out of season while Santos-Jimenez et al. (29) found 84% to 95% of females displayed estrus behavior during the reproductive season, and all of these females went on to ovulate after demonstrating estrus indications. After receiving GnRH, less than 50% of the ewes exhibited estrus signs, and over 80% of them went back to their regular ovulatory activities. In the group receiving eCG, the reaction during seasonal anestrus was comparable.

Statistically there isn't any difference between group 1 and 3 and 4 which quite logic that control group have and stabilized gene expression as it's under normal circumstances without any affect, while ewes treated with sponges were simulated under anestrum phase found to show significantly differs than after given GnRH or eCG. It's found that litter size was significantly different between groups especially between using eCG and GnRH compared to other groups, eCG group gave more twins and triplets maybe due to the absence of dominant follicle out of season this is reported by Dursun (30). It is mostly comparable to other factors that promote granulosa cell proliferation and reduce FSH receptor and steroid hormone expression (31) and plays an important role in ovulation rate and litter size (32, 33). The greatest way to improve the productivity of producing lamb meat is to raise the litter size rates in sheep. In our investigation, there were noteworthy variations in all groups' liter sizes especially group 3 and 4. Litter size was lowest in the control group and MAP group and highest (1.875) in the eCG group. This disagrees with the outcomes of Ayoub et al. (34) which gave lowest with control group (1.3) and highest with progesterone group. Our study's litter size at birth was smaller than that of Gardón et al. (35), who found that ewes treated with MAP sponges had a litter size of 1.4, and Abu EL-Ella et al. (36), who reported that ewes who were not treated had a litter size of 1.1 while our study reported 1 for control group.

In the current study, the gestation period showed around two days longer in GnRH group (152.65 days) than eCG group and control group (150.34 days and 149 days, respectively). These results in accordance with those published by Ayoub et al. (34) using progesterone and PGF2 alpha is almost the same may be due to same climate in Egypt but differs from

others with longer pregnancy duration reaching over 155 days in Iran and Turkey, this variation may be caused by the environment and various sheep breeds. In our study, no significant differences in birth weight among the four groups. However, it was the highest in control group and the lowest in the eCG group. These results were in disagrees with those results obtained by (34, 36) who found that birth weight was high in the treated groups than control group, maybe the difference found was the number of twins and triplets in eCG group may played a role in decreasing the weight of the fetuses upon the number in pregnant wombs.
The current study's results agree with Azawi and Al-Mola (37) suggest that superovulation in Awassi can be induced by using eCG. which may aid in genetic improvement and expand the size of the Awassi sheep breeding population in Iraq. This study revealed that the ewes' synchronization of estrus with vaginal sponge had an impact on the blood levels of progesterone and estrogen (38). In this present study GnRH with combination with MAP did not give as much as eCG group but it gave better female / male ratio 60% while eCG group 43.3% respectively.

Conclusion

The non-breeding season fertility of ewes was positively impacted by all synchronization tested procedures. Combining estrus synchronization utilizing MAP with (eCG) is an easy-to-use method for farmers, requiring little work and handling of the animals especially out of breeding season. Additionally, eCG is a gonadotropin that is sold commercially in Iraq. Our findings were effectively implemented at a private farm with huge flock of sheep grown in semi-extensive settings on farms in Iraq.

Conflict of interest

Authors declare no conflict of interest.

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