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Relevance of Type of Breeding and Single Versus Double Sponge Combined with PMSG on Herri Ewes Estrus Exhibition and Pregnancy Rate

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Abstract: This study was designed to investigate effects of natural mating versus artificial insemination in ewes synchronized for estrus/ovulation by single versus double sponge inserts combined with PMSG. A hundred twenty cyclic Herri ewes were utilized in the study. Ten ewes served as control (C), given no treatment, 58 ewes were designed to be naturally mated at estrus exhibition, 47 of which were inserted with a single sponge impregented in fluorogestone acetate (FGA) for 12 days and given 500 IU PMSG at sponge removal (SSNM-G1) and eleven ewes were given double sponges (DSNM-G2), the first sponge lasted 7 days, removed and replaced by a second sponge for 5 days and at the second sponge removal, 500 IU PMSG was given (i.m.). All ewes were visually observed for estrus signs and at onset of estrus, fertile rams were allowed to mate ewes. Time from sponge removal until estrus exhibition was recorded for each group. The artificially inseminated ewes were randomly allocated into two group, SSAI (G3, n=31 ewes) and DSAI (G4, n=21 ewes) in which ewes were given a single and double sponge inserts, respectively. Animals in G3 and G4 were timed inseminated with chilled fertile semen 48-50 hours after sponge removal. All ewes were diagnosed for pregnancy 30 days post breeding by ultrasound. Proportion of ewes observed in estrus was highest in DSNM ewes (100%), followed by SSNM (91.5 %) compared with C ewes (70%). Mean time interval from sponge removal till estrus was shorter in DSNM (38.1 h) than in SSNM (46.3 h). Likewise, conception and pregnancy rates were higher in DSNM (81.8 and 81.8 %, respectively) than in SSNM (74.4 and 68 %, respectively). Artificial insemination resulted in lower pregnancy rate (42.3 %) with 35.4 % in SSAI and 52.3 % in DSAI ewes. Mean pregnancy rate in control ewes was 50%. In conclusion, inserting two sponges throughout 12 days combined with 500 IU PMSG at sponge removal shortened the interval from sponge withdrawal till exhibiting estrus, increased conception rate and pregnancy rate in Herri ewes.

Key words: Ewes · Estrous Synchronization · FGA · Pregnancy Rate

INTRODUCTION

Improvement in fertility must first come from improved breeding management and the use of assisted reproductive technology (ART) such as estrus synchronization. Estrus synchronization facilitates the use of genetically superior sires through AI. It may also enhance reproductive efficiency by shortening the breeding and lambing seasons. Synchronization of estrus involves the manipulation of the estrous cycle in cycling ewes by induction of estrus in anoestrus ewes so that a large percentage of females come into estrus at a predetermined time. This procedure is known as timed breeding, breeding by appointment or mass mating [1]. For a successful estrous synchronization, the ewes should be closely synchronized with a rapid decline in circulating progesterone concentration as well as synchronous growth and ovulation of a viable follicle. There are two principles of controlling estrus and ovulation in ewe. The first principle is to prolong the life span of the CL, thus delaying estrus. This can be attainable by administering progestin such as a sponge or Controlled Internal Drug Release (CIDR) that mimics the function of the CL. The second principle is to shorten the life of the CL hastening the onset of estrus. This is attainable by administering exogenous luteolytic agents such as prostaglandin $F_{2\alpha}$. However, $PGF_{2\alpha}$ is effective only when a fully developed CL is present approximately between D5 and D7 of the estrous cycle [2].

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Estradiol has been demonstrated to be a good marker for follicular function [3], for both sheep and cattle. In cattle, several studies have shown that healthy antral follicles contain high estradiol concentrations [4] while atretic follicles contain low estradiol concentrations [5] and are less able to release estradiol [6]. The lower values in plasma estradiol concentration found in ewes treated with progestagens would indicate that follicular function might be altered in some of them. The basis for the administration of progestagens is the decrease in LH secretion [7], that prevents the occurrence of estrus, LH surge and ovulation until the withdrawal of the sponge. Maintenance of high levels of progestagen also affects the expression of dominance effects from large follicles and increases follicular turnover [8]. But, sometimes the release of progestagen from the sponges is too low at the end of the treatment; LH is not suppressed to the required level [9] and led to inadequate follicular development, with persistent large follicles [10] that will affect fertility if ovulate [11]. The hypothesis was to evaluate the usefulness of inserting a second sponge within the twelve days combined with 500 IU PMSG at sponge removal on the enhancement of estrus occurrence and subsequent fertility and pregnancy rate in ewes.

MATERIALS AND METHODS

A total of 120 cyclic Herri ewes, 3±5 years of age and 40±45 kg body weight, were used in this study. The experiment was conducted during autumn 2010 at the Agriculture and Veterinary Research Center, Qassim University. The animals were managed under the same conditions on one farm. They were kept under semi-shaded yards, offered barley, alfalfa hay, clean water

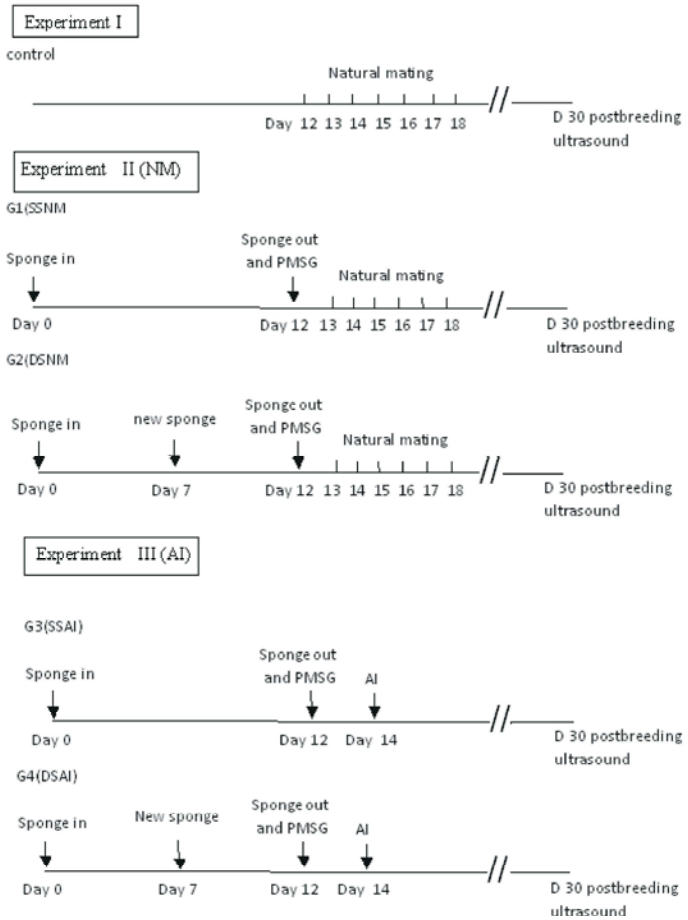

Fig. 1: Sponge-PMSG protocols in experiment I and II. In G2 and G4 sponge was inserted for 12 days and PMSG injection at sponge removal. In G3 and G5, ewes were treated with single vaginal sponges for 7 days and then replaced with new vaginal sponge for the rest time (5 days) and PMSG injection at sponge removal. There was no treatment in the experiment I (control).

and integrated mineral licks. Animals were randomly allocated into 3 experiments. Exp1 (n=10) ewes served as control, exp2 ewes (n=58) served as the naturally mated and subdivided into two groups, the single sponge naturally mated (SSNM) ewes (n=47) and the double sponge naturally mated (DSNM) ewes (n=11). However, ewes were designated for AI and subdivided into two groups, the single sponge AI (SSAI, n=31) and the double sponge AI ewes (DSAI, n=21). Fig. 1 illustrated the design and the time schedule for various animal treatments.

Experiment I (Control): Ten ewes (n=10) served as control receiving no vaginal pessaries and introduced to the males at the same time when ewes of experiment II were introduced to the males.

Experiment II (Natural Mating): Ewes (n=58) were randomly allocated into the following groups; G1 (SSNM, n=47). Ewes were vaginally inserted with a single sponges impregnated with 40 mg of flourogestone acetate (FGA) and left for 12 days. Group 2 (DSNM, n=11): ewes were inserted with a single vaginal sponges for 7 days and then replaced with a new vaginal sponge for the rest 5days. At sponge removal, all ewes were injected (i.m.) with 500 IU of pregnant mare serum gonadotropine (PMSG). At the onset of estrus, natural mating was carried out by the use of fertile rams in a ratio of 1:5. The males were introduced in the herd after pessary removal for a total period of 96 h. the duration from sponge removal until estrus exhibition was recorded. After 30 days of natural mating, pregnancy was confirmed by ultrasound (Esaote, pie medical, Netherlands).

Experiment III (Artificial Insemination): Females (n=52) were randomly allocated into two groups; group 3 (SSAI, n=31), ewes were inserted with a single sponge for 12 days. While, group 4 (DSAI, n=21), ewes were inserted with a sponge 7 days and then replaced by another sponge for the remained 5 days. At sponge removal, all ewes were injected (i.m.) with 500 IU PMSG. Semen from a fertile ram was collected using an artificial vagina. Having determined seminal characteristics, it was diluted with egg yolk-glucose-citrate extender. All ewes were artificially inseminated with fresh diluted semen containing a dose of 200 millions total sperm. Cervical artificial insemination was performed 48-50 hours after sponge removal (Timed AI). After 30 days of artificial insemination, pregnancy was diagnosed by ultrasound.

Statistical Analysis: Data were analysed using a statistical software SPSS release 17.0. Reproductive parameters among 5 group treatments were compared by ANOVA and chi-square. Data are presented as arithmetic mean ± SEM. Significance was considered at $P<0.05$.

RESULTS

Table 1 shows the effect of synchronization treatment on estrus response in the Herri ewes. In the experiment II (natural mating), a total of 54 out of 58 (93.1 %) ewes exhibited estrus. The percentage of ewes observed in estrus was highest in the DSNM (100%), however, only 91.5 % of SSNM ewes exhibited estrus. Although, the difference was not statistically significant ($P>0.05$). Ewes given the second sponge insert have shown estrus shortly (after 38.1 h) after sponge removal as compared with those given a sole sponge (46.3 h). In case of single sponge insert, the estrus exhibition duration scattered between 24-144 h, however inserting a second sponge intensified the estrus duration within short time (<24-48 h). The pregnancy rate after synchronization treatment in the G1 and G2 were 68.0 and 81.8 %, respectively which were higher than control (50%). Despite this enhancement, there was no statistical difference. In experiment III (AI), likewise, the second sponge insert tended to increase pregnancy rate (52.3%) than the single sponge artificially inseminated ewes (35.4%). Comparing natural mating with artificial insemination on the pregnancy rates was in a favor of natural mating (70.6%) which was higher than AI (42.3%).

Distribution of ewes exhibiting estrus over time following sponge removal in experiment II is shown in Figure 2. Forty out of 43 ewes (93 %) exhibited estrus within 72 h of sponge removal in the SSNM ewes, however in ewes given the second sponge 100% exhibited estrus within 48h of sponge removal. No estrus observation was conducted for the AI ewes, since they were designed to be artificially inseminated at predetermined time (48 h after sponge removal). Control ewes exhibited estrus in only 7 out of 10 ewes (70%) with wider time frame of 144 h.

DISCUSSION

In the present study, insertion of a second progesterone sponge not only accelerated the onset of estrus, but it also increased the proportion of estrus occurrence in synchronized ewes. The second dose of

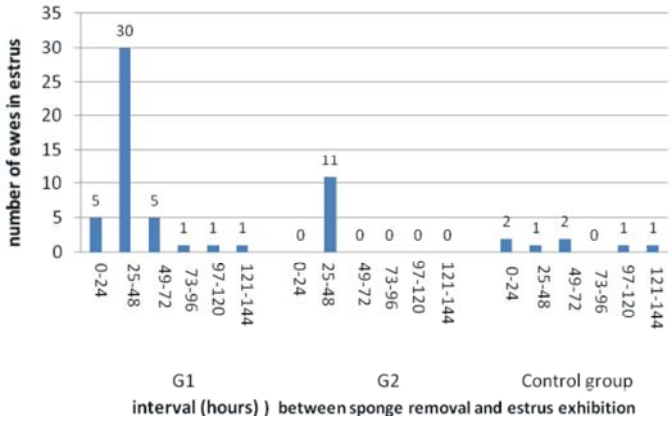

Fig. 2: Distribution of ewes exhibiting estrus over time following synchronization treatment in experiment II

Table 1: Estrus response and pregnancy rate following synchronization treatment in the Herri ewe using natural mating and artificial insemination

Groups	Number	No. of ewes in estrus (%)	Sponge removal to estrus (h)	Conception rate (%)	No. of pregnant ewe (%)
Experiment I Control	10	7 (70.0)	52.0 ± 15.8	71.4	5 (50.0)
Experiment II (NM)					
G1 (SSNM)	47	43 (91.5)^{a}	46.3 ± 3.3^{b}	74.4^{a}	32 (68.0)^{aA}
G2 (DSNM)	11	11 (100)^{a}	38.1 ± 1.4^{a}	81.8^{a}	9 (81.8)^{aA}
Total	58	54 (93.1)	44.05 ± 2.2	75.9	41 (70.6)^{A}
Experiment III (AI)					
G3 (SSAI)	31	-	-	11 (35.4)^{aB}	11 (35.4)^{aB}
G4 (DSAI)	21	-	-	11 (52.3)^{aA}	11 (52.3)^{aA}
Total	52	-	-	22 (42.3)^{B}	22 (42.3)^{B}

^{a,b,c}: Values with different superscripts in the same column and experiment differ significantly at P<0.05, Chi-square and ANOVA.

^{A,B}: Values with different superscripts in the same column between experiments differ significantly at P<0.05, Chi-square.

progesterone might optimized the internal progesterone concentration leading to an optimum peak. When the second sponge insert was removed a sharp decline in peripheral progesterone has been occurred resulting in a better trigger of LH peak. The resultant LH peak might caused better ovulation, resulting in improved pregnancy rate (81.8 %). The use of PMSG in conjunction with intravaginal progestagen treatment was found to efficient for estrus induction synchronization and ovulation [12- 14] in ewes. Similar finding was reported by Dogan and Nur [15] confirming that the double sponge inserts within 14 days accompanied by the injection of PMSG at sponge removal shortened the time duration till estrus than in the single- sponge treated ewes in the non-breeding season.

Concomitantly, other researchers [11, 15-18] found similar interval when used double sponge impregenated with progesterone analogs. When ewes were given progesterone [17] or FGA or MAP intravaginal sponges [16, 18] alone or combined with PMSG [11, 19-22] the estrus response was recorded until 120 h after pessary removal.

These results showed that the time interval to onset estrus in the control and SSNM ewes ranged between 24 to 144 h, whereas in DSNM ewes of this interval has been shortened (25 to 48 h). Therefore, ewes treated with one sponge tended to have longer intervals to estrus than those treated with two sponges. Researchers have reported the onset of estrous to occur within 24-144 h following progestagen or progesterone withdrawal [11, 16- 18]. Ewes came into heat between 18 and 96 h after sponge withdrawal, with the highest incidence of estrous onset occurring between 30 and 60 hours. With respect to distribution of estrous in the present study, ewes was similar to that reported elsewhere [15, 16, 18, 20].

Additionally, the findings of this study demonstrated that some ewes failed to display estrus after estrus synchronization treatment and that was higher in SSNM and control ewes. These findings indicate that there is great variation in the estrus response in Herri ewes after sponge treatment. This variation could be due to various factors and conditions that occur before or during the estrus synchronization treatment. Dogan and Nur [15] demonstrated that, 4 out of 13 ewes did not show any

overt signs of estrous, but were diagnosed pregnant at day 54 after AI. Such a finding is in agreement with a previous report in Boer and indigenous goats [23]. Allison and Robinson [24] suggested that these silent ovulations may be related to inadequate endogenous progesterone levels. Besides, absence of estrus and ovulation may be due to insufficient gonadotrophic hormone released by the pituitary, to a poor response by ovary to the exogenous PMSG or variation in responsiveness of animals to PMSG [25]. Moreover, in the current study only two visual observation periods (07:00- 09:00 and 18:00-20:00 h) were applied, this time schedule might was not enough for visualizing those ewes exhibiting short estrus signs in the rest of the day (24 h.). Failure of estrus detection might be attributed to an inefficient detection scheme and/or the occurrence of true silent ovulations that make it difficult to determine patterns of cyclicity [26].

Ewes given double sponge (DS) either naturally mated or artificially inseminated gave better pregnancy rates than their counterparts give single sponge (SS). It was reported that using one sponge can lead to a decrease in the conception rate after the treatment. The events that bring about ovulation are highly dependent and integrated. Slight changes may disturb the maturation of oocytes and timing of ovulation, which result in impaired developmental competence of oocytes leading to an early embryonic death [27]. In addition, with these changes in timing of ovulation, the chance of normal AI timing is impaired, resulting in fertilization failure and consequently less pregnancy [27]. During this period, the follicular wave pattern was not maintained and a large follicle persisted following spontaneous luteolysis [28]. The sequential relationship of the low progesterone increased the frequency of pulses of LH, a persistent largest follicle increased secretion of esdtradiol-17β and reduced fertility is widely accepted as one of the causes and effects [29]. From these aged large follicles, abnormal ova will ovulate and this may be one of the reasons for low pregnancy rates [29]. This could also be the reason as to why we observed low pregnancy rate in the SS ewes in the present study. One other critical factor that reduced sperm transport can occur after long period of low progesterone treatment [28]. In another study by Shaham-Albalancy *et al*. [30], they reported that concentrations of progesterone before estrus altered endometrial morphology during the subsequent estrous cycle. Low concentrations of progesterone (2.1 to 2.3 ng/mL) during that period increased subsequent secretion of $PGF_{2\alpha}$ in response to oxytocin, as measured by its major metabolite [31]. These effects might lead to lowered embryonic survival and also a decrease in fertility. Thus, low pregnancy rate in single sponge ewes might began very early due to the exposure to lowered progesterone level that may lead to embryonic death.

The physiological explanation of the lower pregnancy rates in AI versus natural mating is due to the predetermined timing of inseminating the ewes designed for AIs. As it is clear that estrus/ovulation occurrence scattered over 144 h in SS ewes, this would impaired the fertilization process in a specific percentage of ewes [32, 33]. However, in double sponge ewes the 52.3 % of pregnancy rate resulting from artificial insemination would be acceptable under the experiment condition. It still comparable to the percentage obtained in SSNM ewes.

CONCLUSION

In conclusion, vaginally insertion of two progestagen sponge in Herri ewes resulted in a shorter intensifying interval from the sponge withdrawal to estrus. This regime increased the possibility of ova fertilization and subsequent pregnancy rate. Further studies warrant to be conducted on large-scale number of ewes from various local breeds inside and outside the breeding season.

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