The “Male Effect” On Estrous Synchronization and Reproductive Characteristics of Female Markhoz Goat during the Breeding Season

Research Article

M.M. Moeini1*, W. Mohammadi Chapdareh1 and M. Souri1

1 Department of Animal Science, Faculty of Agriculture, Razi University, Kermanshah, Iran

Received on: 10 Sep 2014
Revised on: 25 Jan 2015
Accepted on: 15 Feb 2015
Online Published on: Dec 2015

*Correspondence E-mail: mmoeini@razi.ac.ir
© 2015 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran
Online version is available on: www.ijas.ir

ABSTRACT

One hundred female Markhoz goats (42±5 kg) were used to investigate the ‘male effect’ on estrous synchronization and reproductive characteristics of female goat during the breeding season. This was carried out in three separate experiments. In the first experiment, forty female goats were used in 4 groups with different distance from the male goats (i.e. source of pheromone secretion); A1 (1 meter), A2 (12.5 meters), A3 (25 meters) and A4 (50 meters). In the second experiment, thirty female goats were synchronized using CIDR in three groups in the presence or absence of male goats; B1 (the presence of male goat with females during synchronization), B2 (sudden introducing of male goat to females goat during the time of removal of CIDR) and B3 (no male goat during synchronization). In the third experiment, thirty female goats were used in 3 groups; C1 (male goat permanently was presented near to the females during the experiment), C2 (sudden introducing of male to females) and D or control group (no male before mating). The results showed the effect of male goat on estrous synchronization, causing reproductive cycles in early breeding season and increasing incidence of estrous (P<0.05). The male effect improved fertility and kidding rates (P<0.05). Male effect was more pronounced when the distance of unlike sexes decreased (P<0.01). Male effect was also more effective, with sudden introducing of male to female goat (P<0.01). The male effect was more efficient when compared with synchronized female by CIDR in onset of estrous (P<0.05), and increased serum estrogen concentration in the follicular phase (P<0.05).

KEY WORDS estrogen, estrous synchronization, kidding rate, male effect, Markhoz goat.

INTRODUCTION

Reproductive system is controlled and regulated by brain centers and hormones. Central Nervous System (CNS) plays a major role in hormone regulating and secreting and its functions are regulated by certain external factors including light, sound, smell, feed, temperature, pheromones, etc (Hafez and Hafez, 2000; Gelez and Febre-Nysl, 2009). In sheep and goats, exposure to mature males stimulates the reproductive neuroendocrine system of anoestrous females leading to out-of-seasonal ovulation; the so-called ‘male effect’ (Delgadillo et al. 2009). This finding indicates that the continued presence of male is necessary to induce reproductive cycles and increasing secretion of gonadotropin-releasing hormones from the pituitary in female goat. Most of this process is played by pheromones. Pheromones are chemical signals used among cospecifics for social interactions such as sex attraction, mate selection, modulation of neuroendocrine function and individual identification (Okamura and Mori, 2005). A phenomenon referred as "male effect" causes the secretion of GnRH/LH pulses and induces ovulation in mammals in the reproductive and non-
reproductive seasons (Delgadillo et al. 2009; Gelez and Febre-Nysl, 2009). It is known that a pheromone produced by the wax and wool of rams induces ovulation in anestrous ewes (Perkins and Fitzgerald, 1994). Recent studies have revealed that there is a direct relationship between pheromone productions and serum testosterone concentration in male (Gelez and Febre-Nysl, 2009). Females use testosterone-dependent cues to assess potential mates and testosterone concentrations may indicate a male’s overall fitness (Longpre and Katz, 2011). It is established that female goats preferred the male goats with higher testosterone concentration in the mating season. Pheromones are associated with attracting the unlike sex. Pheromones are small molecules, spread rapidly in the environment causing anestrous termination, inducing estrous synchronization and effective fertility (Choen-Tannoudij and Signoret, 1987). The vomero nasal organ (VNO) in small ruminants is morphologically constructed for the detection of pheromones (Booth and Webb, 2011). Pheromones move between the VNO that causes nerve signals, which in turning the central nervous system, causing pulses of GnRH/LH; this makes the end of anestrous, estrous synchronization and fertility (Iwata et al. 2003; Delgadillo et al. 2009). Repeated reports of a maximum ovulate response when females are exposed to the full complement of male socio-sexual stimuli strongly indicate that non-olfactory cues play either a complimentary or synergistic role with chemical cues from the male (Delgadillo et al. 2009). Since pheromones are spread through the air, its concentration in the environment may decrease with increasing distance from the source of pheromones (Mohamadi et al. 2011). These findings suggested that the male effect in husbandry environment may cause differences in reproductive behavior of female goats. Thus, the aims of the present study were 1) to investigate the effect of the sudden introduction of male goat on the sexual behavior of female goat 2) determination of the optimum distance between the male and female goats for successful application of the “male effect” and 3) to assess the efficiency of the intended use of the natural effects of the male goat on the degree of estrous synchronization, reproductive efficiency and synchronizing costs and 4) the determination of the appropriate time for the introducing of male to female goat in order to begin reproductive cycle at early breeding season in Markhoz goat.

**MATERIALS AND METHODS**

This study was conducted in Sanandaj Animal Husbandry Research, Sanandaj, Iran (1373 m altitude with a mean annual temperature of 13.1 °C) at beginning of the breeding season for a total period of 7 weeks (from early August until mid-October 2013).

One hundred Markhoz female goats (3.5 years old, 42±5 kg live weight and with body condition score 3) and 22 male goats were used. Before the start of the experiment female goats were separated from the males for 2 months and isolated of visual auditory and olfactory signs. The females were randomly divided into 10 groups comprised of 10 animals. In the first experiment forty female goats were used in 4 groups with different distances from male goats; A1 (1 meter), A2 (12.5 meters), A3 (25 meters) and A4 (50 meters, Figure 1a). In the second experiment thirty female goats were synchronized by CIDR (EAZI-BREED, New Zealand), in three groups; B1 (the presence of male goat with females during synchronization for 18 days), B2 (sudden introduction of male goat to female goats during the time of removal of CIDR) and B3 (the absence of any male goat during synchronization, Figure 1b). In the third experiment thirty female goats were used in 3 groups; C1 (male goat permanently presented near to females during the experimental period for all time), C2 (sudden introduction of male into females stables after 7 days of experiment) and D or control group (in the absence of any male, Figure 1b). Goats were offered a total mixed ration, according to their body weight, to meet the current estimates of requirements three times daily. Animals had free access to fresh tap water and mineral bricks. Body weight was recorded weekly. During 7 weeks of the experiment, average day length (duration of light time) and temperature were recorded in natural conditions of station. Goats were monitored three times per day to detect their estrous behaviors. Estrous symptoms, including restlessness, making frequent noise, tail moving, reduced feed intake, and edema of the vulva, vaginal mucus appearance and estrous mounting (Mohamadi et al. 2011) and time of induction (onset) of estrous in goats and the time of natural mating during the experiment were recorded. Finally, pregnancy rate, number of kids born and kidding rate were recorded for each treatment at the end of gestation in February and March, respectively. Blood samples were collected 2, 48, and 92 hours after removal of CIDR (B1, B2, B3 groups) or at the time of onset of the first estruscycle (A1, A2, A3, A4, C1 C2, D groups) from jugular vein of each female goat. Serum was separated after centrifugation at 3000 × g for 15 min and stored at -20 °C, until hormone analysis. Estrogen and progesterone concentrations were measured in thawed serum samples using ELISA kits no. ELA-2694 (DRG, Marburg, Germany) and no. ELA-1562 (DRG, Marburg, Germany), respectively.

Male effect response (MER) was measured by the first observation of onset of estrous after 48 hours of introducing of male goat, until 25 days. Female goats were scored according to their response to the introduction of the male goat.
Those showing estrous 48 hrs later received 100 points, thereafter the score was reduced by 4 points per day. Those not showing estrous by 25 d received 0.0 points.

Statistical analysis
All statistical analyses were conducted in SAS (2003) using the Proc FREQ, LOGISTIC, Mixed and GLM with the following model:

\[ y_{ijk} = \mu + \text{Treat}_i + \text{Animal}_j(\text{Treat}_i) + \text{Time}_k + (\text{Treat} \times \text{Time})_{ik} + e_{ijk} \]

Where:
- \( Y_{ijk} \): animal performance.
- \( \mu \): population mean.
- \( \text{Treat}_i \): i treatment effect.
- \( \text{Animal}_j(\text{Treat}_i) \): effect of \( j \) animal in \( i \) treatment.
- \( \text{Time}_k \): effect of \( k \) time.
- \( (\text{Treat} \times \text{Time})_{ik} \): interaction of treatment and time.
- \( e_{ijk} \): residual or error.

The data for kidding rate were modified by application of a log_{10} transformation for homogeneity of variance. Duncan’s multiple range was used to determine significant differences between the means.

RESULTS AND DISCUSSION
The number of goats showing estrous, number of pregnant goats, number of kids born, kidding rate and MER are shown in Table 1. The results showed a positive effect of male goat on sexual behavior and reproductive performance in female goats. Experimental treatment (male effect and CIDR with male effect) improved reproductive traits and had significant effects on the kidding rate (\( \chi^2=783.00, P<0.01 \)). The male effect such as exposure of male goat to females (i.e. higher concentration of male pheromones, sound and viewing), short distance of female from male goats (treatment A1, A2) and sudden introduction of male goat in to females stables (treatment C2) caused better estrous synchronizing and kidding rate when compared with control group (P<0.05).
Also by measuring MER or rapid response of female to male effects (treatments A1, A2, C2), it was observed that the more females showed their reproductive cycle and time of onset of estrous in a short time (P<0.01, Table 1). The male effect caused considerable differences in blood estrogen concentrations (Table 2). There were significant differences in estrogen concentration among experimental groups during estrous (P<0.05). The goats in A1, A2, B1, B2 and C2 groups (the “male effect”) had highest estrogen concentrations (Table 2) resulting in an increase in the number of kids born (Table 1). Among the groups of male effect, in which females were exposed to male goat (i.e. higher concentration of male pheromones, sound and viewing), the animals with short distance from male goats (groups A1, A2) and sudden introducing of male goat into females (groups C2, B2) showed significantly higher concentrations of the estradiol-17 beta hormone (P<0.05, Table 2). Those groups which had higher estrogen concentrations had also higher progesterone concentrations at the reproductive cycles (Table 3). Treatments which synchronized estrous by CIDR (B1, B2, and B3) and the “male effect” treatments, progesterone concentration in blood serum were more than other groups (Table 3). These changes in certain hormones concentrations are associated with improved fertility performance (Scaramezzi et al. 2006). So indirectly we can suppose that the male effect and e.g. (higher concentrations of pheromone in the husbandry environment) induced reproductive cycle and more pulses of GnRH/LH which caused an increase in estrogen and progesterone (Table2). Therefore, we would observe an increase in estrous synchronize and more kidding rates (i.e. more ovulation) (Table 1). Hamada et al. (1996) showed that exposure of female goats to the male goat hair leads to LH pulses in less than 2 minutes (Hamada et al. 1996). Increase infrequency of LH pulse, has been demonstrated to affect the GnRH/LH pulse insheep (Hamada et al. 1996; Over et al. 1990).

These researchers found that there was on able cause of this behavior was pheromone in male goat hair. Delgadillo et al. (2009) showed in ewes, 6 h of contact with rams induce a rapid increase in LH secretion, but is not enough to provoke ovulation, and LH secretion returns to pre-stimulation levels after the male is removed. Pheromone with the scattering through the air that stimulates the neuro-endocrine system in females, followed by release pulses of GnRH/LH stimulate andinduce ovarian activity and starting of reproductive cycle.

The result of this experiment showed that the “male effect” affects the estrogen concentrations and other reproductive parameters by distance changes. The short distance between female and male goats (treatment A1, A2) was more effective than longer distance (treatment A3, A4) probably because of the amount of pheromone concentration in the environment (Mohamadi et al. 2011). Several studies have suggested that continuous presence of the male is required to maximize the endocrine or ovulatory responses of females to the male stimulus (Delgadillo et al. 2009; Hawken and Beard, 2009). Signoret et al. (1982) and Hawken and Beard (2009) determined that 20% of ewes ovulated when exposed to teasers for 24 h, and that this increased to 51% for 4 days of exposure, and to 61% for 13 days of exposure.

Four hours of daily contact with sexually active males is sufficient to stimulate ovulatory activity in female goats (Bedos et al. 2010). This effect was not due to the presence of olfactory cues from the males remaining in the pens, but it depends on the active presence of males for 4 hours (Bedos et al. 2010). The relationship between duration of male contact and the efficacy of the male stimulus is also evident in goats, where exposure to males for 16 h per day for 10 days induced ovulation in only 19% of goats, while continuous exposure induced ovulation in 95% of females (Walkden-Brown et al. 1993a).

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Groups</th>
<th>MER1</th>
<th>Number of goats showing estrous</th>
<th>Number of pregnant goats</th>
<th>Number of kids born</th>
<th>Kidding rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First experiment</td>
<td>A1</td>
<td>76.2±7.13b</td>
<td>10a</td>
<td>9a</td>
<td>11a</td>
<td>105a</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>61.9±7.13b</td>
<td>10a</td>
<td>9a</td>
<td>10a</td>
<td>95a</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>26.4±7.13d</td>
<td>10a</td>
<td>8ab</td>
<td>9a</td>
<td>85a</td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>4.3±7.13d</td>
<td>5b</td>
<td>4b</td>
<td>5b</td>
<td>45a</td>
</tr>
<tr>
<td>Second experiment</td>
<td>B1</td>
<td>89.4±7.13b</td>
<td>9a</td>
<td>8ab</td>
<td>10a</td>
<td>90a</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>99.1±7.13a</td>
<td>10a</td>
<td>9a</td>
<td>11a</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>65.2±7.13b</td>
<td>8ab</td>
<td>6ab</td>
<td>7b</td>
<td>65ab</td>
</tr>
<tr>
<td>Third experiment</td>
<td>C1</td>
<td>37.9±7.13</td>
<td>7a</td>
<td>7a</td>
<td>8a</td>
<td>75ab</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>64.8±7.13a</td>
<td>10a</td>
<td>9a</td>
<td>11a</td>
<td>100a</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>11.0±7.13d</td>
<td>5b</td>
<td>4c</td>
<td>5b</td>
<td>45a</td>
</tr>
</tbody>
</table>

The means within the same column with at least one common letter, do not have significant difference (P>0.05). A1, A2, C1, C2: male effect with changes of distances; B1, B2: CIDR with the male effect; B3: CIDR; C1, C2: male effect; D: control and C2 and B2: sudden introducing of male.

<table>
<thead>
<tr>
<th>Number of goats showing estrous</th>
<th>Number of pregnant goats</th>
<th>Number of kids born</th>
<th>Kidding rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>9a</td>
<td>11a</td>
<td>105a</td>
</tr>
<tr>
<td>10a</td>
<td>9a</td>
<td>10a</td>
<td>95a</td>
</tr>
<tr>
<td>10a</td>
<td>8ab</td>
<td>9a</td>
<td>85a</td>
</tr>
<tr>
<td>5b</td>
<td>4b</td>
<td>5b</td>
<td>45a</td>
</tr>
<tr>
<td>9a</td>
<td>8ab</td>
<td>10a</td>
<td>90a</td>
</tr>
<tr>
<td>9a</td>
<td>11a</td>
<td>10a</td>
<td>100a</td>
</tr>
<tr>
<td>6ab</td>
<td>7b</td>
<td>65ab</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>7a</td>
<td>8a</td>
<td>75ab</td>
</tr>
<tr>
<td>9a</td>
<td>11a</td>
<td>10a</td>
<td>100a</td>
</tr>
<tr>
<td>5b</td>
<td>4c</td>
<td>5b</td>
<td>45a</td>
</tr>
</tbody>
</table>

Table 1: Response to male effect; number of goats in estrous, number of pregnant goats, number of kids born and kidding rate in each group (n=10 in all cases)
However, Rivas-Muñoz et al. (2007) reported that 95% of female goats ovulated in response to sexually active males, while only 15% responded to quiescent males indicating that, in goats at least, there is an interaction between sexual activity of the male and duration of daily exposure on the percentage of females responding to the male effect (Rivas-Muñoz et al. 2007).

In the present study, similar to results obtained by Hawken and Beard (2009) and Delgadillo et al. (2009) the male effect had effectively stimulated on the reproductive behavior in the females.

If we consider the four-week test period, the results of starting estrous in goats treatments A1, A2 and C2 were more effective than of test of Delgadillo et al. (2009) (Figures 1 and 2). Meanwhile, the goats which showed signs of estrous is continuous and long-term in control group and were less than a fore mentioned treatments, which implies endorsement male effect on the onset of estrous in female goat (P<0.01) and our results show significant differences between experimental groups in the early weeks of the experiment (Table 1 and Figures 1 and 2). This phenomenon indicates that the male effect not only caused an early time of onset of estrous accelerates but also increased estrous synchronization (P<0.05) thus increasing the chances of pregnancy and kidding rate (Table 1). The male effect treatments such as expose male goat with females (i.e. higher concentration of male pheromones, sound and viewing) in short distance of female from male goats (groups A1, A2) and sudden introduction of male goat in females group (C2).
and the permanent presence of males in females group (C1), showed a greater number of goats estrous in a shorter time (Figures 1 and 2) and greater numbers of kids born (Table 1) therefore, long-term male effects on fertility is positive and was consistent with the findings Gillies et al. (2004) and Perkins and Fitzgerald (1994).

While in group D, a gentler slope was observed over a longer period. It is observed in Figure 1 that less distance between males and females (i.e. higher concentration of male pheromones, sound and seeing) in treatment A1 during the first 2 weeks have resumed 100 percent of their reproductive cycles and have shown signs of estrous and had significant difference with other treatments (P<0.01). While the treatments had a greater distance (A4) there was observed a longer time and gentler slopes with fewer estrous goats (Table 1 and Figure 1). This is important in relation to reproductive management of farm animals, as Gokuldas et al. (2010) showed that if Buffalo during postnatal constantly be exposed male effect, will resume faster their reproductive cycle, silent ovulation decreases and the first service will increase the chances of conception (Gokuldas et al. 2010).

The onset of estrous at the third week was observed in (D) (Figure 3). It occurred because of beginning of the breeding season due to climatic conditions in this area around the third week. The collected data showed that the breeding season of Markhoz goat beginning at early October, when the temperature decrease to 24 °C and time of brightness reduce to 12 hours. Twenty days after initiation of the experiment (20 August) estrous in the control group (D) was observed (Figure 3). This was could be due to the effects of climate (temperature and day length). We conclude with are view of the status of onset of estrous in goats examined (Figure 4) that between 7 to 20 days of starting the test before the breeding season, male effects on females were meaningful and began the reproductive cycle. Furthermore, the curve in Figure 4 has a higher peak of male effect in the second week compared with peak season effect (the second wave of the curve).
So in addition to the male effect increasing the onset of estrous, estrous synchronization power was more effective than the effect of the season.

In other words, we can say that the male effect leads to the early start of the reproductive season. Hawken and Baird (2009) showed that short-term exposure (24 h) of ewes and rams prior to the breeding season led to animals that were rapidly estrous synchronized (Hawken and Beard, 2009). Perkins and Fitzgerald (1994) found that when ewes exposed to rams in time of transition from the non-reproductive season to breeding season it stimulates estrous behavior in the females so that 17 to 24 days after exposure ewes showed estrous (Perkins and Fitzgerald, 1994). According to the researchers; olfactory signals would increase due to the further concentrations of pheromones and decreasing the distance from male (Hafez and Hafez, 2000). In fact female goats preferred the male goats with higher testosterone levels in the mating season. Studies indicate that females were potentially able to detect signals from male behavior (Longpre and Katz, 2011). The specific changes in reproductive activity in males and females at breeding season or non-reproductive season depend on the nutrition level (Walkden-Brown, 1994; Delgadillo et al. 2009; Mohamadi et al. 2011). Exposure of short term rams with ewes, the female tend to male animal (Choen-Tannoudij and Signoret, 1987) although exposure of males with females does not necessarily require previous separation (Cushwa et al. 1992). Bedos et al. (2010) study showed that, 4 h of daily contact with sexually active males is sufficient to stimulate ovulatory activity in anovulatory goats and this effect is not due to the presence of olfactory cues from the males remaining in the pens (Bedos et al. 2010).

Also Delgadillo et al. (2009) showed that the entering a male goat in to a group of females which are at the end of the non-reproductive season, not only brings advances to the beginning of the breeding season for several days but also synchronizes their reproductive cycles. This phenomenon of male-induced synchrony is also evident in cyclic goats with a high proportion of females showing estrous within 6 days of male exposure (Choen-Tannoudij and Signoret, 1987; Delgadillo et al. 2009). Under temperate and subtropical latitudes, ewes and goats display a reproductive seasonal pattern and their sexual activity during the anestrous period can be stimulated and synchronized by the introduction of males in the group, which is called the “male effect”. The response of females to the male effect in the middle of the anestrous season is weak or absent. It may be due to a low quality stimulus provided by the male which is, as the females, in seasonal rest (Delgadillo et al. 2006) in female goats, there is strong evidence that the genotypic effect of seasonality on the responsiveness to the male effect can be circumvented by using sexually active males (Delgadillo et al. 2006). In the highly seasonal Saanen breed, a full ovulatory response was initially obtained after stimulation with males that had been treated with artificial light and melatonin during late anestrous, even though cyclicity could not be maintained if the females did not also receive a photoperiodic and melatonin treatment (Chemineau et al. 1986). In the seasonal Mexican mixed breed, the use of males rendered sexually active by a photoperiodic treatment leads to ovulation and conception during periods of anestrous when untreated males elicit no response (Delgadillo et al. 2006; Flores et al. 2000). These observations support those of Walkden-Brown et al. (1993b), who reported that seasonal variation in the responsiveness of females to the male effect in northern Australia can be avoided by using bucks in which reproductive function had been stimulated nutritionally (Delgadillo et al. 2009). However, the results of the present experiments (Figures 3 and 4) confirm that it can be employed males effect during the breeding season for estrus synchronization and increase oestrus in the early breeding season and kidding rate with natural control. It is recommended that male effect in subsequent experiments with environmental and climatic conditions (i.e. 25 August) is consistent with the estrous synchronization and kidding rate in Iran.

Results of the using CIDR to synchronize estrous are shown in Figure 5. Considering that after 18 days in female genital tract and two days after taking CIDR, the estrous signs were observed in females. So, as expected, estrous synchronization lasted in female goats 20±0.5 days. However, significant differences were observed between treatments in the number of on set of estrous goats (P<0.05).
Like third test, sudden appearance or entrance of a male goat in a females group in a day before CIDR-removal (treatment B2) synchronouse strous was 100% and increased kidding rate (Figure 5 and Table 1). B3 treatment had only synchronized by CIDR (non male effect) only70% of the goats showed signs of estrous after CIDR-removal (Figure 5).

![Figure 5](image-url)

**Figure 5** Onset of estrous after removal of CIDR in female Markhoz goats at breeding season (%)

The superiority of the method using male effects is evident, when compared with CIDR group only (P<0.05). Because “male effect” caused on set of estrous in females after 15 days, lead to increasing number of female goats to cycle activation. Also the problems caused by the use of CIDR were eliminated. These problems include costs for purchasing CIDR and adverse events including. Infection of the uterus and vagina and protrusion or falling CIDR of genital tract. Infectious agents using CIDR can reduce reproductive performance in females (Lamb and Larson, 2004).

**CONCLUSION**

The present study indicates an improved reproductive efficiency particularly early estrous in breeding season, synchronized estrous and kidding rate by male effect. The male effect, compared with CIDR was more successful in inducing estrous activity and cyclist. Thus using males during the breeding season for early onset of estrous and estrous synchronization might increase the kidding rate in Markhoz goat. The male effect was more efficient when compared with synchronized female by CIDR in onset of estrous and also increased serum estrogen concentration in Markhoz goat during the follicular phase.

**REFERENCES**


