

TRANS-CERVICAL ARTIFICIAL INSEMINATION IN EWES DURING OUT OF BREEDING SEASON

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The study was conducted to determine the effect of transcervical insemination with frozen thawed semen in synchronized ewes at the anestrus phase. A total of 100 ewes (crossbred of Akkaraman, Sakız, Border Leichestre, Lincoln, Ile de France) at the Animal Science Research Center of the Ankara University were used. Estrus was synchronized with progestagen-impregnated vaginal sponges for 14–16 days in January. Following synchronisation of oestrus, 750 IU PMSG was injected per ewe. Ewes were artificially inseminated with frozen semen after 3–4 h intra-cervical administration 50–100 mg of PGE₁ analogue (misoprostol) for each. Lambing, infertility and prolificacy rates were found as 44.87%, 55.12% and 91.02% in natural-mated group and 68.18%, 31.81% and 109.09% in the misoprostol group, respectively. Fertility rate was numerically increased in the misoprostol group compared to the control. Infertility rate was also numerically decreased in the treated group in comparison with the control ewes. The results indicated that acceptable fertilization and pregnancy rates could be obtained in estrous synchronized and transcervically inseminated ewes after misoprostol treatment.

Key words: sheep; estrus synchronization; prostaglandin E₁; transcervical insemination

ТРАНСЦЕРВИКАЛНО ОСЕМЕНУВАЊЕ КАЈ ОВЦИ ВОН СЕЗОНА НА ПАРЕЊЕ

Студијата беше спроведена за да се утврди ефектот на осемнување преку грлото на матката (трансцервикално осемнување) со замрзната сперма, одмрзнувана на собна температура кај синхронизирани овци во фаза на анеструс. Беа искористени вкупно 100 овци (мелези на Akkaraman, Sakız, Border Leichestre, Lincoln, Ile de France) во Истражувачкиот центар за животински науки при Универзитетот во Анкара. Осемнувањето беше синхронизирано со прогестаген со помош на вагинални сунѓери за период од 14–16 дена во месец јануари. По синхронизација на еструсот, кај секоја овца беше аплицирано по 750 IU PMSG. Овците беа вештачки осемнети со замрзната сперма по 3–4 часовно вршење на осемнување преку грлото на матката со 50–100 mg на PGE₁ аналоген (мизопростол) за секоја од нив. Процентите на јагнење, јаловост и плодност беа 44,87%, 55,12% и 91,02% кај природно спарената група и 68,18%, 31,81% и 109,09% кај групата во која е користен мизопростол, соодветно. Процентот на плодност е бројно зголемен кај групата во која е користен мизопростол во споредба со контролната група. Процентот на јаловост исто така беше бројно намален кај третираната група, во споредба со контролираните овци. Резултатите покажуваат дека би можеле да се добијат прифатливи проценти на осемнување и бременост кај синхронизирани овци, осемнети преку грлото на матката по третманот со мизопростол.

Клучни зборови: овци; синхронизација на еструс; простагландин E₁; трансцервикално осемнување

INTRODUCTION

Based on the FAO statistics, there are 21,794,500 heads of sheep in Turkey (FAO, 2010). Almost all Turkish sheep breeds show seasonal breeding which is regulated by day length. It is commonly approved that artificial insemination

(AI) of sheep with frozen semen plays an important role in genetic improvement through extensive exploitation of superior germ plasm. Trans-cervical AI which is one of semen deposition techniques is known as the Guelph system for trans-cervical intrauterine AI (GS-TCAI). It has given greater success in cervical penetration and lambing rate fol-

lowing insemination (Naqvi et al., 1998, Leethongdee et al., 2005; Lucy et al., 2010). But TCAI has own limitation due to the complex anatomy of the ovine cervix (The ovine cervix is long, fibrous, tubular convoluted organ and also its lumen has corkscrew-like nature), with its eccentrically located six annular rings and small ring openings which both generally prevent the passage of the AI pipette through the body of the uterus (Wulster-Radcliffe et al., 2004; Kershaw et al., 2005; Leethongdee et al., 2005). Some factors such as breed, age, parity and physiological state can affect the length of the ovine cervix. The mean length of the cervical canal has been described as, 6.5, 5.5 and 6.7 cm respectively and ranged from 5.7 to 10 cm with high individual variability (Kershaw et al., 2005). The rate of TCAI achievement in sheep may vary between 57–76% (Windsor 1995) and 10–15% (Kershaw et al. 2005) depending on breeds. Such different success of TCAI among individual ewes may be explained by great variation in cervical anatomy among animals (Kershaw et al., 2005) so that there are considerable differences between species, even each of sheep regarding the complexity of cervical rings, organization of the inner and outer orifices, length and complexity of the cervical lumen and anatomical relationships with the uterine body and vagina (Leethongdee et al., 2005). Estrus synchronization allows for parturition at suitable times to take advantage of niche markets, feed supplies, labor, and rising price trends (Whitley and Jackson, 2004). Recent researches showed that intravaginal application a PGE1 analogue such as Misoprostol can induce cervical ripening without adversary effect on sperm transport and fertility in ewes (Barbas et al, 2001; Barbas et al, 2003). The aim of this study is to investigate the use of a PGE1 analogue for cervical dilataion in order to induce trans-cervical artificial insemination by using frozen-thawed semen in estrus synchronized ewes.

MATERIALS AND METHODS

Animals and management

This experiment was conducted during out of the breeding season for the local sheep breeds (January-May 2010) at the Agricultural Research and Application Farm of Ankara University, Faculty of Agriculture which is located at 39° 61 N and 32° 68 E latitude. The altitude of the area and average rainfall are 1065 m. and 414 kg/m² per annum, respectively. A total of 100 multiparous

and lactating cross-breed ewes (obtained from Akkaraman, Ile de France, Border Leichester, Lincoln and Chios) kept in a semi-intensive system were used. Ewes were regularly fed with alfalfa hay and supplemented with 0.5 g concentrates containing 2400 Kcal ME / Kg and 13% CP. Animals had free access to fresh drinking water. The composition of the basal diet was adjusted according to the NRC sheep requirements (National Research Council, 1985) and met or exceeded the nutritional recommendations of Pulina and Bencini (2004). The animal body condition score at insemination was 3–4 based on a scale of 1–5 (Russel, 1991).

Estrus synchronization

All ewes were treated with intravaginal sponges containing 40 mg progesteron (40 mg FGA[®] Fluorogestone Acetate, Intervet Boxmeer, Holland) on 11th of January for 14 days and an i.m injection of 750 IU PMSG (Intervet Boxmeer, Holland) at sponge removal, in order to induce and synchronize heat. In the trial group, each of ewes received 5 mg of a prostaglandin E1 analogue (Misoprostol, Aris; Ali Raif İlaç Sanayi, Istanbul-Turkey), after 48 hr of PMSG administration. For that purpose, half of the pills of Cytotec[®] (200 µg of Misoprostol each) was powdered and mixed to 1–2 drop of glycerol (glycerol approximately 87%, Merck; it was just for diluting and embedding into cotton and also for facilitating the retention of Misoprostol by the vaginal wall). As cervical ripening needs 4–6 h after Misoprostol application, Misoprostol was administered 48 h after sponge removal (Leethongdee et al., 2007; Goldberg et al., 2001). Ewes were checked for estrus before Misoprostol application by using teaser rams and visual inspection of vagina. Ewes without signs of heat have not received misoprostol. In misoprostol treated ewes, the vagina was washed by physiological saline (0.9% NaCl, placebo) after misoprostol impregnated cotton removal.

All ewes were restrained in a good position and their cervix was viewed using a speculum fitted with a light source and were inseminated once. Frozen-thawed semen was provided from Bahri Dağdaş International Agricultural Research Institute of the Ministry of Food, Agriculture and Livestock (Konya, Turkey). Prior to insemination, straws were thawed at 37 °C for 15 s. At the time of AI, average motility, live/death and abnormal spermatozoon percentages were found as 79%, 5% and 5.65% respectively. The assesment was performed by using a light microscopy (400 ×). The

insemination dose contained about 120×10^6 spermatozoa per 0.25 ml.

Pregnancy, lambing, and fecundity rates were calculated as follows (Zelege et al., 2005):

pregnancy rate = (ewes lambed/ewes inseminated) \times 100

lambing rate = (lambs born / ewes inseminated) \times 100

fecundity rate = (lambs born / ewes lambed) \times 100

Statistical analysis

The chi-square test (χ^2) has been used with a confidence interval of 95% and p values less than 0.05 considered as significant difference. The analysis was done using the Minitab software (version 11.5) (Gavcar, 2009).

RESULTS AND DISCUSSION

The reproductive performances for all synchronisation treatments are presented in Table 1 and 2. As it is shown in Table 1, the lambed ewe number following AI and the natural mating were found as $n = 15$ and $n = 35\%$ respectively. There was no significant difference ($\chi^2 = 3.73$; $df = 1$; P-value = 0.053; Table 2) regarding fertility (%), fecundity (%) and prolificacy (%) between artificially inseminated with frozen-thawed semen ewes and those naturally mated. Fertility rate was numerically increased in the treated group compared to the control animals. Therefore infertility rate was numerically decreased in the treated group (Table 2).

Estrus synchronization in ewes and cows focuses on the manipulation of either the luteal or the follicular phase of the estrous cycle. The opportunity for control is greater during the luteal phase in ewes, which is of longer duration and more responsive to manipulation. Strategies can be employed to extend the luteal phase by supplying exogenous progesterone or to shorten this phase by prematurely regressing the existing corpora lutea. In this regard not only synchronization but also provision of acceptable levels of fertility upon AI or natural mating are important views (Turk et al., 2008). Many studies showed the importance of administering PMSG to obtain a more predictable and compact oestrus or ovulation even though cyclic ewes or those which are expected to demonstrate estrus shortly after intravaginal progestagen withdrawal without administration of PMSG (Zelege et al., 2005).

Table 1

Reproductive outcomes of ewes inseminated with frozen – thawed semen and naturally after synchronised oestrus by progestagen and PMSG

Variable	AI	Natural mating	Total
Number of ewes	22	78	100
Number of ewe lambed	15	35	50
Infertile ewe number	7	43	50
Single lambing	6	–	–
Twin lambing	7	–	–
Number of lamb born	24	71	95

Table 2

Reproductive performance of artificially inseminated and naturally mated ewes after progesterone and PMSG treatment (%)

Reproductive traits	AI	Natural mating
Fertility	68.18	44.87
Infertility rate	31.81	55.12
Fecundity	109.09	91.02
Prolificacy	160	202.85

$\chi^2 = 3.73$; $df = 1$; P-value = 0.053

Misoprostol is used commonly and effectively to induce cervical softening and cervical relaxation at parturition in women and vaginal application of Misoprostol enhanced intrauterine insemination in women. Cervical ripening at parturition can be induced with 100 mg of intravaginal Misoprostol. The recommended dose for cervical ripening in women during the first trimester of pregnancy is 400 mg (Leethongdee et al., 2006). Naqvi et al. (1998) reported 22.7% of lambing rate in ewes inseminated by using this technique. In our study, similar lambing rate (22.7%) is obtained as the above-mentioned research. In a study performed by Timurkan and Yildiz (2005), the conception rates in Hamdani ewes were synchronized by a different dose of PMSG hormone (500 IU, 600 IU, 750 IU and control) were found as 90.62%, 93.75%, 100% and 79.4%, respectively. They concluded that administration of progestogens (FGA), using intravaginal sponges, and PMSG to Hamdani ewes in the breeding season appears to be effective in the synchronization of oestrus and parturition and in increasing mean litter size and rates of

pregnancy. It was revealed that the treatment of ewes with PMSG increased the number of follicles and therefore raised the twinning and triplet rates (Timurkan and Yildiz, 2005). The percentage of oestrus and pregnancy rates in ewes treated with 30 mg of Cronolone (FGA) + 700 IU of PMSG was reported 90% and 76.4% by Tumen et al. (1992). When ewes treated by 40 mg of FGA+750 IU of PMSG the oestrus and pregnancy rates were 100% and 96%, respectively (Gulyuz and Kozat, 1995).

There are contradictory results on the pregnancy and oestrus response rate by different researchers which can be explained by the differences in body condition, breed of experimental animals, management systems and the season in which the studies were executed (Timurkan and Yildiz 2005; Horoz et al. 2003; Krajinović et al., 1985; Dominguez Fdez-Tajerina et al., 1991; Gokcen et al., 1992; Zeleke et al., 2005).

In conclusion, the current study shows that a successful TCAI and reasonable lambing rate can be achieved in progesterone + PMSG treated ewes by using frozen-thawed semen.

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