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ORIGINAL ARTICLE

The Determination of Plasma Progesterone, Estradiol-17β Hormone Levels in Ghezel Sheep treated with CIDR and Various Doses of PMSG during the Breeding Season

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ABSTRACT
The aim of the present study was to evaluate different doses of PMSG on Plasma Progesterone, estradiol-17β hormone levels in Ghezel ewes synchronized with controlled internal drug release device (CIDR) during the breeding season period. A total of 28 ewes was used in this experiment. All animals were divided randomly into four groups then a single intramuscular (IM) injection of PMSG (group 2, 350 IU, n=7; group 3, 450 IU, n=7; group 4, 550 IU, n= 7), group 1 (n=7) was made apart from 1 ml normal saline solution which was used as a control group at the time of CIDR removal. Blood samples were collected from the jugular vein at the days of CIDR insert removal, Estrus after PMSG treatment and 30 the day of pregnancy for the determination of plasma E2 and P4 concentrations. There were significant differences (P<0.05) between the treatment groups and the control group regarding the plasma E2 levels at days of CIDR insert and estrus. Plasma E2 levels at day estrus were 13 pg/ml, 16.9 pg/ml, 17.6 pg/ml and 10.9 pg/ml in groups 1, 2, 3 and the control group, respectively. Differences between the treated and the control animals in the Plasma P4 levels at day estrus and 30 the day of pregnancy were significant (P<0.05). Plasma P4 levels at 30 th day of pregnancy were 0.94 ng/ml , 1.1 ng/ml, 1.24 ng/ml and 0.82 ng/ml in groups 1, 2, 3 and the control group, respectively.
Key words: Ghezel ewe, Pregnant Mare Serum Gonadotropin (PMSG), Estradiol-17β (E_2), Progesterone (P_4)

INTRODUCTION

Applications of exogenous hormones for increased reproductive performance in domestic ewes usually focus on estrous synchronization. Estrous synchronization in goats and sheep is achieved by control of the luteal phase of the estrous cycle, either by providing exogenous P_4 or by inducing premature luteolysis. Progestogens, one of the types of hormone used for synchronization in ewes, can be given by oral administration, subcutaneous, or intravaginal insertion. Blood progesterone levels increase within 1-4 h according to the route of administration. Traditionally, sponges impregnated with either native P_4 or analogues and then inserted into the vagina for a given period of time have been used. Afterwards, PMSG may be injected 48-54 h after CIDR removal. Controlled internal drug release (CIDR) devices, consisting of a nylon core surrounded by a silicone elastomer that is impregnated with P_4, have been developed as an alternative. Controlled internal drug release silicone devices can release limited levels of progesterone for a longer time than is recommended for synchronization (1). Pregnant mare serum gonadotropin (PMSG) is used for induction of ovulation and estrus is used most effectively for synchronization during the breeding season. In addition, pregnant mare serum gonadotropin (PMSG) has been found to increase ovulation rate and twinning in a dose related manner. Between all endocrine approaches to increase reproductive performance, administration of PMSG is more usual than others. Injection of PMSG at the end of the progestogens treatment causes more precise synchronization of oestrus in small ruminants (2).Yu *et al*. recorded a high correlation between the development of follicles with serum progesterone (P_4) and estradiol (E_2) concentrations. Previous studies reported that progesterone concentration might affect the follicular size. Estradiol concentration was also reported to have a positive correlation with the number of large follicles during the estrus cycle (3).The aim of this study was to determine the influences of various doses of PMSGon E_2 and P_4 hormone levels inGhezel ewes inseminated by fresh semen.

MATERIALS AND METHODS

Location, animals and treatments

This experiment was carried out at breeding station of Ghezel sheep in Miyandoab in West Azarbayjan province in Iran in breeding season, from September to October. The site is located at 46°6′E latitude, 36°58′N longitude and 1314m from the sea level in the center of the plain areas which ends at south front of Lake Urmia. The annual rainfall in this region ranges from 250 to 300 mm . A total of 28 Ghezel ewes 2-4 years-old and weighing 45-55 kg, were used in this study. CIDR were inserted into vagina of the ewes for 14 days. In group 1 (n=7), group 2 (n=7) and group 3 (n=7) 350 IU, 450 IU and 550 IU of PMSG was administered, respectively, at the time of CIDR removal. In the control group (n=7), ewes were injected with 1ml normal saline solution at sponge removal to act as untreated controls.

Blood samples

After the CIDR implantation (day 0), a series of blood samples was collected at days of CIDR insert removal, Estrus after PMSG treatment and 30 th day of pregnancy. Blood samples were obtained from a jugular vein using vacutainer vials and centrifuged immediately after collection at 3000 rpm for 10 minutes at 4°C. The blood plasma was then stored at -20°C until assayed. Concentrations of progesterone were determined by ELISA kit (Monobind®; USA) with 0.1 ng/ml sensitivity. The plasma estradiol (E_2) concentration was measured by ELISA kit (DRG International, GmbH, USA) with 0.625 pg/mL sensitivity.

Statistics

Statistical analyses on the concentration of estradiol and progesterone were performed on a microcomputer using Statistical Package for Social Science (SPSS) programme (version 20.0). Data were analyzed through analysis of variance (ANOVA) with significant difference level of $P<0.05$.

RESULTS AND DISCUSSION

Table I shows the mean plasma estradiol-17β concentration at days CIDR insert removal and estrus after PMSG treatment. Estradiol-17β levels were significantly different at days of CIDR insert and estrus between groups ($P<0.05$). Average estradiol-17β (E_2) levels at day CIDR insert were 1.3±0.21, 2.1±0.58, 1.2±0.42 and 1.4±0.39 pg/ml in the Normal saline, 350, 450 and 550 IU PMSG groups, respectively. The difference betweenthe groupsin estradiol-17β concentration at day CIDR insert maybe related to thedifferentstagesof theestrouscycle the experimental animals used in this study .Differences in estradiol-17β concentration at day CIDR removal were not statistically significant between groups ($P>0.05$). Average estradiol-17β concentration(2.92 pg/ml) at day CIDR removal similar to previously reported (4).

Probably, CIDR block the ovarian steroidogenesis due to the presence of progesterone in the serum. High P_4 concentration would inhibit FSH thus, prevent development of ovarian follicles, ovulation and estrus (5).

CIDRs (**C**ontrolled **I**nternal **D**rug **R**elease) are an intravaginal progesterone insert used in the beef cattle, dairy cattle, goat and sheep industries. The progesterone is released at a controlled rate into the bloodstream after insertion. In all species, CIDRs are used for the synchronization of estrus. In this study all treatment groups showed reduction in P_4 levels after CIDR insert. Hussein and Ababneh reported that this phenomenon would reset the hypothalamic-pituitary-ovarian to regress persistent follicles and recruit new healthy follicles. Estradiol (E_2) is considered as a good marker of follicular quality. The number of large follicles and E_2 concentrations were positively correlated during the estrus cycle (6).

Average estradiol-17β levels at day estrus (48 hours after administration of PMSG) were 10.9±1.34, 13±2.12, 16.9±1.49 and 17.6±2.47 pg/ml in the Normal saline, 350, 450 and 550 i.u. PMSG groups, respectively. This result also can be attributed to increased litter size related in the increase of PMSG dose. However, the mean estradiol-17β levels in groups 450 and 550 IU PMSG was higher than in the 350 i.u. PMSG and control groups and increased depending on increasing PMSG dose. These results are in accordance with those reported by some researchers (7,8,9) with regard to effect of administered PMSG on estradiol-17β levels.

In the present study Ghezel ewes have received different doses of PMSG following 13-day progesterone treatment.Pregnant Mare Serum Gonadotrophin (PMSG) can be substituted for both LH and Follicle Stimulating Hormone (FSH) of the anterior pituitary gland which stimulate development of the ovarian follicle. Pregnant Mare Serum Gonadotrophin injection may increase the number of ovulations and consequently, it leads to increase plasma estradiol-17βconcentration in synchronized ewes (5).

Table II shows the mean progesterone concentration (ng/ml) per examination at days of CIDR insert removal, Estrus after PMSG treatment and 30th day of pregnancy. Mean progesterone concentration of the animals in groups 1, 2, 3, and 4 at day of CIDR insert were found to be 2.5±1.40, 2.5±0.99, 2.8±1.16 and 2.7±1.38ng/ml, respectively. There was no significant difference between the treated groups and also

between the treated groups and the control group (P>0.05). The mean concentration of blood progesterone at day of CIDR removal were 0.89±0.22, 0.75±0.14, 0.82±0.15 and 0.80±0.15ng/ml for ewes in the 1,2,3 and 4 groups, respectively. No significant differences in term of mean progesterone concentration were recorded between groups. The results on progesterone concentration at days of CIDR insert removal for treated groups were consistent with that reported by some other researchers (1,4,8,10, 11).

Menegatos et al. [12] conducted a study in which Chios breed ewes were treated for 14 days with either MAP intravaginal sponges or subcutaneous progesterone implants, followed by administration of 500 IU PMSG at the time of withdrawal. During treatment circulating progesterone levels were 1.02±0.12 and 1.43±0.16 for the sponge and implant group , respectively, while at day of progesterone withdrawal progesterone levels dropped to 0.2 ng/ml in both groups (Fig. 1).

Vinoles(13)reported that, use of a CIDR device resulted in peak plasma P4 levels of 2.1 ng/ml within 24 h of application, and relatively stable levels between days 1 and 13 (1.9 ng/ml).Peripheral blood progesterone concentrations indicate the reproductive physiology of animals. Progestogens and $PGF_{2\alpha}$or their analogues were used in order to condense parturition and oestrus of the ewes in the breeding season.In small ruminants, estrus synchronization was achieved either by extending the cycle with exogenous progesterone or its analog progestagen or reducing the length of the luteal phase of the estrus cycle with prostaglandin ($F_{2\alpha}$) (1). These synchronization regimes were orally implanted or intravaginal sponges insertion. These devices would exert negative feedback on Luteinizing Hormone (LH) secretion that inhibited the endocrine events and lead to the maturation of preovulatory follicles and ovulation (2).Intravaginal devices with progesterone or progestogens are the most commonly used procedures which can be combined with gonadotrophin hormone to increase ovulatory efficiency and ovulation rate. Present study showed significantly higher concentrations of P_4 and E_2 for PMSG administration at CIDR removal.

Mean progesterone concentration at day estrus were 0.39±0.03, 0.37±0.05, 0.40±0.12 and 0.45±0.06ng/ml in groups 1, 2, 3 and the control group, respectively. This results were consistent with that reported by Cunningham et al (11).Cunningham et al. conducted a study in which Cheviot ewes were used to determine levels of progesterone in the plasma during the oestrous cycle .*In the study of* Cunningham et al. Plasma progesterone levels increased progressively during the period 15 to 9 days before oestrus to a mean level of about 2-5 ng/ml, and remained at this level for several days (Fig. 2). By 2 days before oestrus, the mean plasma progesterone concentration had fallen to 1.42 ng/ml, and on the following day it had dropped to <0.5 ng/ml. It remained at this low level until after Day 2 of the cycle, and then again showed a progressive rise. This pattern of changes in progesterone concentration during the oestrous cycle of sheep is similar to that found in peripheral plasma by other workers (Stabenfeldt et al., 1969; Thorburn et al.,1969) and reflects the activity of the corpus luteum(11). The data presented above confirm that, in the cyclic ewe, plasma progesterone values fall to very low levels on the day before oestrus, also PMSG, when injected immediately after the removal of CIDR increased the rate of ovulation hence, increasing multiple births and litter size (14).Mean progesterone concentration at 30th day of pregnancy were 0.82±0.27, 0.94±0.21, 1.10±0.19 and 1.24±0.18 ng/ml in groups 1, 2, 3 and 4, respectively. Mean progesterone concentration in group 4 with injection dose of 550 IU (1.24±0.18 ng/ml) was the highest value between all groups (P<0.05).

This value in groups 3 (1.10±0.19 ng/ml) and 2 (0.94±0.21ng/ml) were higher than group I (0.82±0.27ng/ml) (P<0.05). Some papers reported that administration of 300 IU PMSG and less than was not sufficient to stimulate additional follicular development or was weak for some breeds response (7,9,15). In the present study we observed a PMSG-*dose-dependent increase* in *progesterone levels* between groups at 30 th day of pregnancy. This results was similar to the previous findings of Oyedipe et al. (15).Oyedipe et al. (15) conducted a study in which Yankasa ewes were used to determine the effect of dose of pregnant mare serum gonadotrophin on estrus parameters, ovulation rate and peripheral progesterone concentrations. Ovulation rates (based on number of corpora lutea) averaged 1.0±0.0, 1.3±0.3, 2.0±0.0, 5.5±0.5 and 7.0±1.2 for ewes treated with 0, 250, 500,750 and 1000 IU PMSG, respectively.The results indicate the possibility to increase ovulation rates in ewes with PMSG and to establish a direct relationship between PMSG dosage, ovulation rate and plasma progesterone concentrations.

TABLE I: Mean estradiol-17β concentration (pg/ml) per examination at days of CIDR insert removal and Estrus after PMSG treatment

Groups	1 (Normal saline)	2 (350 IU PMSG)	3 (450 IU PMSG)	4 (550 IU PMSG)
Number of ewes	7	7	7	7
Day of CIDR insert	1.3±0.21[b]	2.1±0.58[a]	1.2±0.42[b]	1.4±0.39[b]
Day of CIDR removal	3±1.47[a]	2.6±0.97[a]	2.8±0.86[a]	3.3±1.44[a]
Day of estrus	10.9±1.34[c]	13±2.12[b]	16.9±1.49[a]	17.6±2.47[a]

Different superscripts in a row show significant difference ($p<0.05$)

TABLE II: Mean Progesterone concentration (ng/ml) per examination at days of CIDR insert removal, Estrus after PMSG treatment and 30 th day of pregnancy

Groups	1 (Normal saline)	2 (350 IU PMSG)	3 (450 IU PMSG)	4 (550 IU PMSG)
Number of ewes	7	7	7	7
Day of CIDR insert	2.5±1.40[a]	2.5±0.99[a]	2.8±1.16[a]	2.7±1.38[a]
Day of CIDR removal	0.89±0.22[a]	0.75±0.14[a]	0.82±0.15[a]	0.80±0.15[a]
Day of estrus	0.39±0.03[b]	0.37±0.05[ab]	0.40±0.12[ab]	0.45±0.06[a]
30 th day of pregnancy	0.82±0.27[c]	0.94±0.21[ab]	1.10±0.19[ab]	1.24±0.18[a]

Different superscripts in a row show significant difference ($p<0.05$)

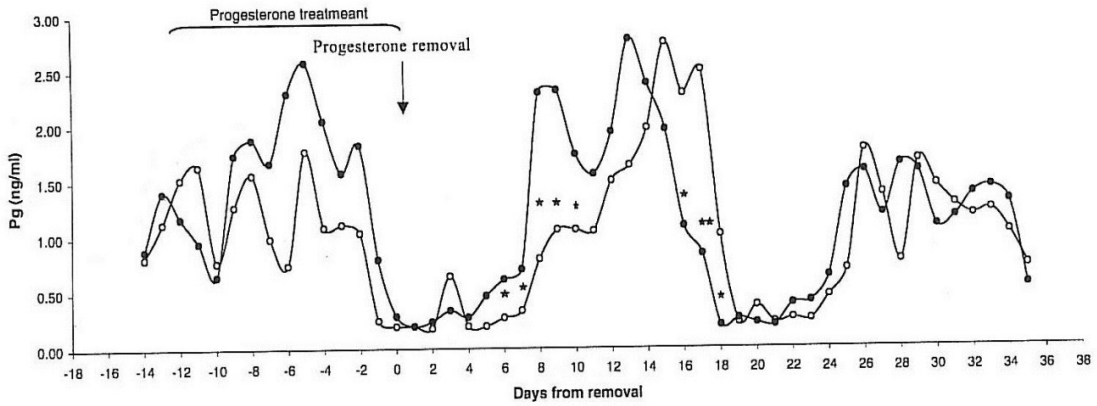

Fig.1. Progesterone concentration in ewes treated with MAP sponges (○) or progesterone implants (●) during the 14-day treatment period and up to 35 days after treatment withdrawal.

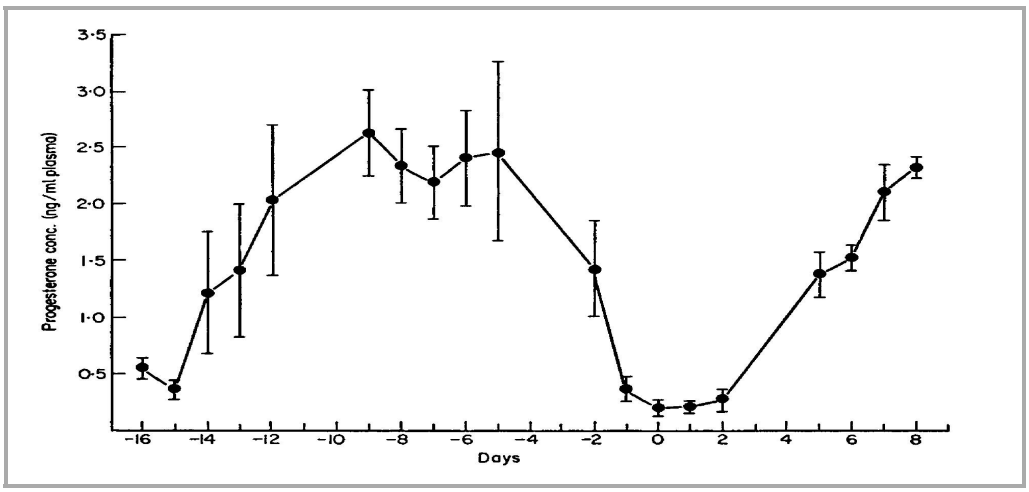

Fig .2. Plasma progesterone levels during the estrous cycle of eight ewes. Each point represents the mean for three to eight animals and vertical bars indicate the S.E.M. Day 0 = day of estrus.

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