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Full Length Research Paper

Effects of equine chorionic gonadotropin (eCG) administration and flushing on reproductive performance in Nadooshan goats of Iran

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An experiment was conducted on Nadooshan goats of Iran, during the breeding season, to evaluate the effectiveness of flushing and equine chorionic gonadotropin (eCG) treatment on reproductive traits. Ninety-two intact does with an average live weight of 25.11 ± 3.933 kg were randomly divided into four equal experimental groups. Controlled internal drug release device (CIDR) were inserted for 18 days in all groups. Experimental groups consist of: 1- Control (C); 2- eCG (600 IU; E); 3- eCG (600 IU) + Flushing (E+F); and 4- Flushing (F). Does in two last groups (E and E+F) were injected intramuscularly, at the time of CIDR withdrawal, with 600 IU of eCG. The first group (C) received no treatment and served as a control. All animals were submitted to pasture and mineral licks and groups E+F and F additionally received flushing ration. Pregnancy rate in group F was more than the control group ($p < 0.05$). Twinning rates and kid crops in groups E+F and F were significantly higher than control group ($p < 0.05$). Time of onset of estrus decreased in groups with eCG administration (E and E+F). Therefore, it is concluded that the flushing especially coupled with the eCG treatment can improve reproductive performance in Nadooshan goats treated with CIDR during the breeding season.

Key words: eCG (PMSG), flushing, reproductive performance, goat.

INTRODUCTION

The most economically important trait in sheep production is reproduction and it can be manipulated using hormonal treatments (Astan et al., 2007). There are several methods for reproduction improving in ewes, often aim to increase the proportion of ewes having multiple ovulations, and thereby increase lambing rate (Akoz et al., 2006). Nutrition is one of the important factors that have effect on many reproductive phases in animals such as estrus and ovulation (Smith, 1991; Somchita et al., 2007). Providing supplemental nutrition to ewes prior to breeding, known as flushing, has been used in sheep to increase ovulation rate and embryo survival (Abu El-Ella, 2006). In sheep and goats, flushing could stimulate ovulation (Molle et al., 1995; Landau and Molle, 1997; Vionles, 2003) and increase the number of lambs that born per lambing. Supplemental protein, energy and minerals can increase the number of viable embryos from a mating (Coop, 1996).

The nutritional effects on the reproductive performance of female small ruminants classified to: (i) the effect of body weight (BW) and body condition (BC) is termed "static" effect (in contrast with "dynamic"), that is, induced by different levels of stable metabolism, if BW, BC, or better, body composition have been kept steady at least 3 weeks before estrus; (ii) changes in BW or BC occurring during 3 weeks before estrus, that affect reproduction, are termed "dynamic" effects; and (iii) "immediate nutrient" effects were exerted by shift in nutrient supply less than ten days pre-ovulation and does not have any effect on body weight and body condition score. During this period, proper nutrient supply is critical for follicular development (Landau and Molle, 1997).

Immediate nutrient effect for energy compartment of food has more important than protein. The effects of

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Table 1. Four experimental Nadooshan does groups in the present study.

Group	CIDR	Flushing	eCG
C	+	-	-
E	+	-	+
E+F	+	+	+
F	+	+	-

+ = Group has received treatment; - = group has not received treatment.
C = control; E = eCG; E+F = eCG + flushing; and F = flushing groups.
eCG = Equine chorionic gonadotropin; CIDR = controlled internal drug release device.

energy and protein cannot be separated completely because in ruminant about 35% of glucose requirement can be provided from amino acids; therefore increase in protein to some extent can be increase in glucose (Landau and Molle, 1997). Glucose is a major source of energy for the ovary (Rabiee and Lean, 2000; Rabiee et al., 1997a, b, 1999).

Recent information indicates that the effect of increased level of nutrition on hypothalamus-pituitary function is exhibited within few days (Abu El-Ella, 2006). In general, nutritional status can affect gonadotropins secretion (Rhind and Schanbacher, 1991; Anson et al., 1991; Boland et al., 2001), progesterone (Parr, 1992; McEvoy et al., 1995; Boland et al., 2001), oestradiol (Payan et al., 1991), insulin (Rhind and Schanbacher, 1991; Downing et al., 1995_a) and growth hormone (Rhind and Schanbacher, 1991; Dowining et al., 1995_a), ovulation rate (Smith, 1991; Parr, 1992; Coop, 1996; Somchita et al., 2007) and ovum quality (Jabbour et al., 1991; Dowining et al., 1995_a; McEvoy et al., 1997).

It has been shown that the administration of gonadotropins such as equine chorionic gonadotropin (eCG) stimulates follicular growth and increases ovulation rate and fertility and induces a tighter synchrony of ovulation in both anestrous and cycling sheep (Dogan and Nur, 2006). Injection of eCG after progesterone treatment, increase estrus response, conception rate and percentage of multiple births from the induced ovulation. In Iranian fat-tailed ewes, injection of eCG, especially in high dosage (600 vs. 400 IU at the time of CIDR removal) increases twinning and lambing rates (Zare Shahneh et al., 2006).

The main objective of this experiment was to studying 1) effects of each of eCG treatment and flushing individual effects, and 2) simultaneous effects of eCG and flushing on important reproductive traits in Nadooshan goats. According to our hypothesis, increase in ovulation rate by eCG infusion is required to provide sufficient nutrients, especially glucose. Therefore, we expected that when these two treatments (eCG and flushing) are used together, better results will be obtained.

MATERIALS AND METHODS

Location, animals and treatments

This experiment was carried out at Yazd province in Iran on Nadooshan goats in breeding season, from September to October of 2005. Ninety-two mature, cycling Nadooshan does weighting (means ± SD) 25.11 ± 3.933 kg were allocated randomly into four equal experimental groups: 1- Control (C); 2- eCG (600 IU; E); 3- eCG (600 IU) + Flushing (E+F); and 4- Flushing (F). All groups were treated with controlled internal drug release device (CIDR) for 18 days. Immediately after CIDR removal, injection of eCG were given to groups E and E+F intramuscularly. At the same time, same volumes of physiologic serum were injected into groups C and F. All animals were submitted to pasture and mineral licks and groups E+F and F additionally received flushing ration (250 g/day/doe barely grain) from two weeks before introducing bucks for three weeks (Table 1).

Mating, estrus and pregnancy detection and kidding records

Three fertile Nadooshan bucks were introduced to each group (12 bucks totally) twice per day (0800 - 1100 and 1700 - 2000 h), starting about 24 h after CIDR withdrawal, and left with them for estrus detection and natural mating. Does were observed continuously during the 3 h when bucks were introduced to them and their matings were recorded.

For detection of pregnant does, an ultrasound device was used externally on day 45 after mating. For prevention of pregnancy toxicity in late pregnancy, all does received additional 250 g/day/doe barely grain. During the kidding time, number, sex and weight of kids were recorded. None of the does littered more than two kids, therefore twinning rate was considered instead of prolificacy. Kids were weighed after drying and before eating colostrum. Kid crop was calculated as: (total weight of born kids)/(total number of tested does).

Statistical analysis

Continuous data (estrus onset, birth weight and kid crop) were analyzed by the PROC GLM procedure and discrete variables by the LOGISTIC procedure of SAS (Der, 2002). Probability values of less than 0.05 were considered significant. Continuous data are expressed as mean ± SD and discrete data are expressed as mean.

RESULTS AND DISCUSSION

Mean estrus onset (mean intervals from progesterone resource withdrawal to onset of estrus) in groups that were treated with eCG (groups E and E+F) were significantly less than two other groups (32.6 h vs. 45.7 h; $P < 0.05$) (Figure 1). Estrus incidence rates were not different significantly among groups. Group F had significantly ($P < 0.05$) higher pregnancy rate than the control group. Flushing, especially coupled with eCG administration, increased twinning rate ($p < 0.05$; Table 2). Flushing and eCG did not have any effects on birth weight of kids, but the kid crops in groups E+F and F were significantly higher ($P < 0.05$) than groups C and E (Figure 2).

In does tested in the present study, Body Condition Score (BCS) range was 1 - 3 (basis on 1 - 5 scoring sys-

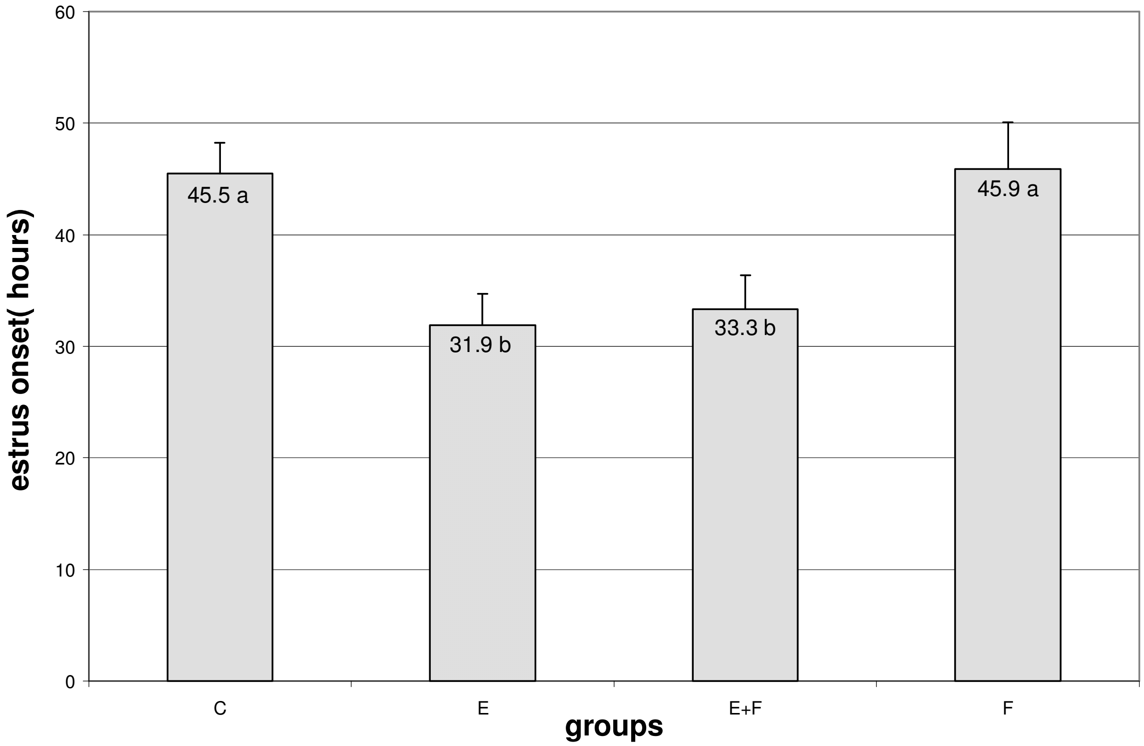

Figure 1. Effects of flushing and eCG treatment on estrus onset. C = control; E = eCG; E+F = eCG + flushing; and F = flushing groups. [ab]Mean with different letters are significantly different ($p < 0.05$).

Table 2. Effects of eCG treatment and flushing on estrus incidence, pregnancy and twinning rate in Nadooshan goats.

Groups	n	Estrus incidence rate (%)	Pregnancy rate (%)	Twinning rate (%)
C	23	95.7 (22/23)	82.6 (19/23)	0 (0/19)
E	23	91.3 (21/23)	91.3 (21/23)	4.8 (1/21)
E+F	23	91.3 (21/23)	91.3 (21/23)	42.9* (9/21)
F	23	100 (23/23)	100 * (23/23)	21.7* (5/23)

*Significantly different from control ($p < 0.05$).
C = control; E = eCG; E+F = eCG + flushing; and F = flushing groups.

tem) and range of age was 2 - 6 years. Does that had higher BCS exhibited estrus earlier than the others, but ages of does did not have any significant effects on estrus onset. Pregnancy rate was not significantly affected by BCS of does but was significantly the highest in four year old does. Twinning rate increased parallel to age and BCS ascendance. The kid crops were the highest in the does with BCS 3 (data not shown).

The results of present study show that the eCG treatment can shorten the mean estrus onset. Pendleton et al. (1992) observed 31.5 h in average time to onset of estrus in dairy goats treated with 6 mg of Norgestomet implant for 14 days plus follicle stimulating hormone (FSH) administrating at 48 h before implant removal. Similarly, Avendano et al. (2003) found an average time to onset of estrus of 24.6 and 16.8 h using ear implants containing 3 and 6 mg Norgestomet, respectively, placed for 11 days plus 500 IU eCG administered 24 h before implant removal. Dogan et al. (2005) observed 20.6 h in mean time to onset of estrus in hair goats treated with intravaginal progestagen sponges for 11 days plus 500 IU PMSG was injected in 9th day. Greyling et al. (2002) in their study about superovulation and embryo transfer in South African Boer and indigenous feral goats, treated donor animal with 20 mg FSH injection and recipients with CIDR for 17 days. The interval from cessation of treatment to estrus for donor was 42.0 and 33.5 h and recipient 27.2 and 28.5 h for Boer and indigenous does, respectively. Fonseca et al. (2005) also stated 41.6 and 53.6 h in mean time to onset of estrus in non-lactating

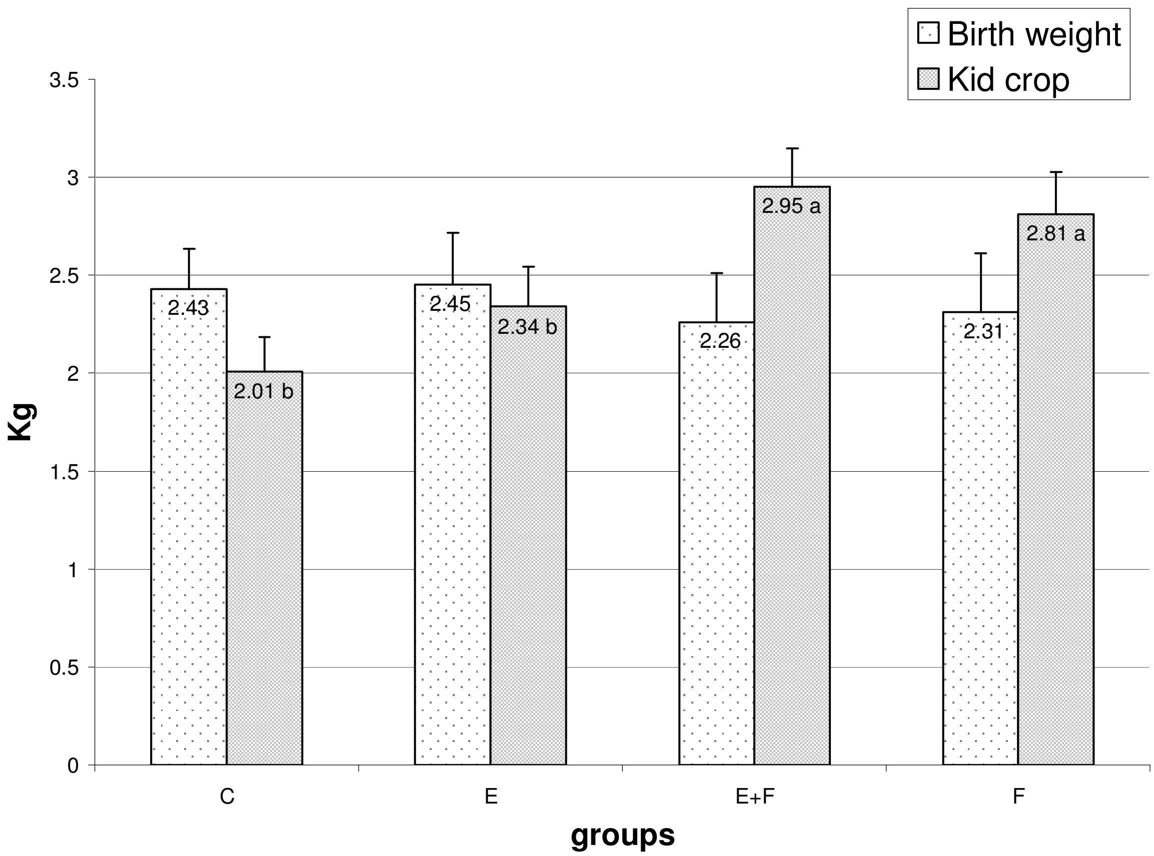

Figure 2. Effects of flushing and eCG treatment on birth weight (kg) and kid crop. C = control; E = eCG; E+F = eCG + flushing; and F = flushing groups. [ab]Mean with different letters are significantly different ($p < 0.05$).

goats treated with intravaginal sponge containing 60 mg medroxyprogesterone acetate for 6 and 9 days respectively, plus 200 IU eCG and 22.5 µg cloprostenol 24 h before sponge removal. In addition, they also indicated that goats have 1 - 4 follicular waves in each estrus cycle and does that did not received eCG needed longer time for start of follicular waves and thereby needed more time for onset of estrus. Likewise, Dogan and Nur (2006) indicated that PMSG administration had an important effect on the formation of compact estrous and ovulation. Regueiro et al. (1999) reported that the treatment of dairy goat with intravaginal sponges contained mederoxyprogesterone acetate during a period of 14 days and injection of 500 IU eCG at the time of sponge withdrawal, caused the average time between sponge withdrawal and standing heat to become significantly shorter in the eCG group than the control group (34.5 vs. 42.9 h). In our study, the interval to onset of estrus in the eCG-treated groups was shorter than the other groups. It seems that the eCG increases the predictability (reduction of the variation in response) of the intervals from implant removal to estrus and ovulation. The reduction of interval to onset of estrus is due to the action of eCG on follicular development (Kridli and Al-Khetib, 2006). eCG mainly acts like FSH and it is reported that FSH hastens speed of follicular growth in early follicle maturation (Viñoles, 2003), and therefore can shorten interval of progestagen withdrawal to estrus onset. The administration of eCG (500 IU) before progestagen sponge removal resulted in a shorter interval to estrus than control and increased the ovulation rate (Ali, 2007).

In this study, does that had more desirable BCS (higher than 2) exhibited estrus earlier than the others. Wildeus and More (1995) reported that does with inappropriate BCS nursing their kids, were unable give a proper response to flushing and thus, did not show estrus very well. BCS represents nutrition status of animals. Paula et al. (2005) stated that only fifty per cent of adult Saanen crossbred goats that were fed 70% maintenance for 6 months, exhibited estrus and ovulation. Furthermore, Rondina et al. (2005) reported that a lower number of local adult goats submitted for 9 weeks to 54% of maintenance level of nutrition were in estrus.

The eCG administration and the flushing do not have any effects on estrus incidence rate. This result indicates effectiveness of CIDR solely to estrus synchronization during breeding season.

We observed that flushing, as expected, increased pregnancy rate and it was highest in four year old does, but eCG treatment do not have any significant effect on pregnancy rate. Optimum body weight as static effect and nutrition as dynamic effect could improve pregnancy rate (Wildeus and More, 1995). Body condition and flushing may enhance conception rate, ovulation rate, or both simultaneously (Landau and Molle, 1997). Age of dams is one of the effecting factors on pregnancy rate. In beef cattle, age and previous reproductive performance (whether the female had failed to conceive or be detected in estrus during the previous breeding season) significantly affected first-service conception rate, as did the interaction between the two factors (Azzam et al., 1989).

In this study we observed that the flushing, especially coupled with eCG treatment, increased twinning rate. Flushing was reported to increase ovulation rate in Rasa Aragonesa ewes (Landau and Molle, 1997; Meza-Herrera et al., 2007). Nutrition is one of the exogenous inputs that affect reproductive functions at different levels of the hypothalamic–hypophyseal–gonadal axis (Meza-Herrera, 2007). Intake of additional protein increases the Gonadotropin Realizing Hormone (GnRH) secretion and thereby increases circulation gonadotropin levels and that, in turn, increases ovulation rate. On the other hand, decreasing of energy supply cause lower GnRH, luteinizing hormone (LH) and FSH levels in circulation (Downing and Scaramuzzi, 1991). Thus, inadequate energy and protein in diet causes negative balance of energy for physiological activities like movement, lactation and reproduction (Evans and Maxwell, 1989). Evaluating on the role of static body condition (BC) and its interaction with a short-term protein supplementation (PL) on secretion of metabolic hormones [growth hormone (GH), insulin and insulin-like growth factor-1 (IGF-1)], as well as on secretion of LH and progesterone (P4) was carried out in sheep and results indicate a predominance of the static component of nutrition on sheep metabolic hormone responses, GH and IGF-1, with no effect of short-term PL on secretion of pituitary and ovarian hormones as well as luteal number and activity (Meza-Herrera, 2007).

Various metabolites and metabolic hormones, includeing glucose, NEFA, insulin, and IGF-I, have been implicated as factors affecting ovarian steroidogenesis, follicular dynamics, and *in vitro* oocyte development (Patton et al., 2007). Although changes in blood metabolites and metabolic hormones have been used to evaluate potential cause-and-effect relationships between nutrition and reproduction without obvious conclusions, compelling arguments have been made suggesting that several nutritionally related signals serve as messengers fundamental to the process of reproduction. Glucose is one of the most important metabolic substrates required for proper function of the reproductive processes (Hess et al., 2005). It is one of the important factors that affecting hypothalamic–hypophyseal–gonadal axis in blood glucose, which is affected by nutrition (Downing et al., 1995_b). Glucose is a major source of energy for the ovary (Rabiee and Lean, 2000; Rabiee et al., $1997_{a \& b}$, 1999) and the primary metabolic fuel used by the central nervous system, so that inadequate availability of utilizable glucose reduces hypothalamic release of GnRH. Some reviewers suggest that the role of glucose in mediating nutritional control of reproduction is permissive rather than causative. Low blood glucose may be detected by the hypothalamus in a threshold-dependent manner such that GnRH secretion will be impaired if glucose availability is inadequate. Stimulation beyond the threshold to promote GnRH secretion is possible by increasing gluconeogenesis via dietary manipulation (Hess et al., 2005) (Figure 3). However, excess energy intake in sheep will lead to significant reductions in progesterone concentrations. Nutrition, unless radically changed, will have little effect on gonadotropin concentrations in ruminants, and this is in contrast to the situation for pigs and for primates, where very short-term nutritional changes manifest themselves in altered gonadotropin secretion (Boland et al., 2001).

Starch in ruminant diets supplies glucogenic precursors, either indirectly via propionate production in the rumen or directly through glucose absorption from the small intestine. In dairy cows the ruminal digestion of starch varies among foodstuffs: ranging between 0·60 for maize and sorghum, to 0·90 for wheat and barley (Abramson et al., 2005). Supplemental feed in present study was barley grain.

Usefulness of eCG administration in sheep estrus induction and synchronization programs is well established. It is applied following progestagen removal to improve follicular development and to increase ovulation rate and multiple births (Kridli and Al-Khetib, 2006), but in the present study, solitary eCG treatment do not significantly affect twinning rate. On the contrary, administration of eCG in ewes, especially in high dosage (600 vs. 400 IU at the time of CIDR removal), increases twinning and lambing rates (Zare Shahneh et al., 2006).

In the present study, ascendance of dam's BCS and age increased twinning rate significantly. Several studies have demonstrated that increasing of does body weight in mating increased twinning rate (Amoah et al., 1996; Downing and Scaramuzzi, 1991). Anwar and Ahmad (1999) reported that in Teddy goats of Pakistan, mean ovulation rate increased as the animal advanced in age.

Kid crop is the important economic indicator for goat's holders. Kid crop depend on two factors: prolificacy (that is discussed earlier as twinning rate) and birth weight. Although in this study, the flushing and eCG treatment did not have any effects on birth weight of kids, it affected kid crop via twinning rate. Moreover, kid crop in does with better BCS was higher. We have previously reported that the eCG treatment increases lamb crop in seasonally anestrous Iranian fat-tailed ewes (5.35 vs. 1.4 kg/tested ewe in group treated with 600 IU eCG and control group, respectively) (Zare Shahneh et al., 2006).

Conclusion

According to the previous studies, gonadotropins like eCG

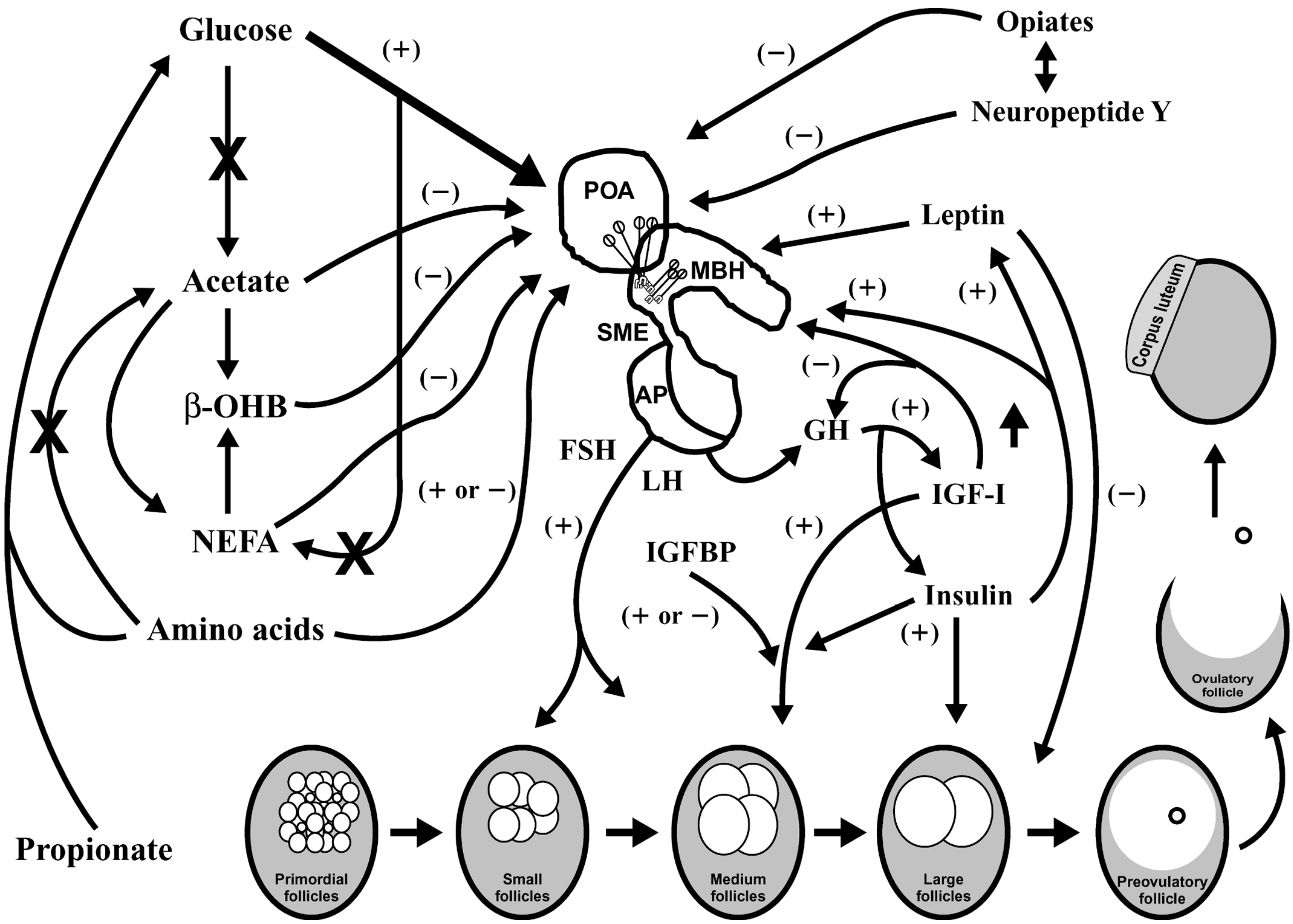

Figure 3. Metabolic regulation of physiological processes associated with estrus in the postpartum beef cow. *β*- OHB = *β*-hydroxybutyrate (Hess et al., 2005).

increase ovulation rates and therefore twinning rates and lambing or kidding rates. On the other hand, flushing also has the same effects. Our theory for this experiment was when gonadotropin eCG and flushing were used together, this effect would be more apparent. According to our hypothesis, if nutrient requirement for ovary particularly glucose (that is main fuel for ovary) is not supplied adequately, eCG administration will not be useful very much. In other word, increasing in ovulation rate by eCG infusion requires the provision of sufficient nutrients, especially glucose. Therefore, we expected that simultaneous application of the two treatments (eCG and flushing) could lead to better results.

When estrus synchronization is carried out as in management practice for accretion of reproductive performance, environmental and nutritional status are very important to achieve maximum efficiency. This study shows that during the breeding season in Nadooshan goats of Iran treated with progesterone (CIDR), the solely eCG treatment (600 IU) does not have any effect on reproductive performance, but the supplemental feeding with barley grain (250 g/day/doe) more than maintenance requirements around mating (flushing), especially coupled with eCG administration increases reproductive efficiency.

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