



Effect of hCG, GnRH and progesterone on reproductive performance and serum progesterone concentration in estrus synchronized ewes

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Abstract

This study aimed to evaluate the effect of human chorionic gonadotropin (hCG), GnRH, and progesterone administration on day 4 following synchronization on reproductive performance of estrus-synchronized ewes. In addition, this study aimed to determine the relationship between these hormonal treatments and serum progesterone concentrations. Thirty-two ewes were synchronized using intravaginal sponges. At sponge withdrawal, 625 IU of (PMSG) pregnant mare serum gonadotropin was administered to each ewe. Ewes were randomly set into four groups of eight. On day 4 post-natural mating, treatment was as follows: Group G1 (control) received normal saline, Group G2 received 500 IU hCG, Group G3 received 0.0042 µg GnRH, and Group G4 received 50 mg progesterone. Pregnancy was diagnosed by a rectal ultrasound probe. On days 0, 12, 22, and 32, blood samples were collected for - ELISA. Results; showed that reproductive performance including estrus response, conception rate, and litter size improved in the ewes treated with progesterone, hCG or GnRH in comparison with control ewes. In the treatment groups, estrus appeared and the conception rate reached 100%, in contrast to 90% in the control group. Moreover, the likelihood of twin births was 4.58 times greater in the hCG group. Progesterone levels increased in all groups on days 12, 22, and 32 post-synchronization. Notably, the hCG treated ewes demonstrated the most pronounced progesterone increases on days 22 and 32, followed by the GnRH and progesterone groups. This study concluded that the applied HCG on day 4th post estrus synchronization stimulated the early formation of corpus luteum and increased reproductive performance, litter size and progesterone levels.

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Introduction

In small ruminants, early embryonic death during the first few weeks of pregnancy is a major factor contributing to reduced fertility (1). A deficiency in corpus luteum function leads to insufficient progesterone secretion, which can compromise early embryonic development. This hormonal imbalance disrupts the signals necessary for maternal recognition of pregnancy and weakens luteotrophic support during the early luteal phase (2). In addition, a diminished LH surge may impair follicular maturation, leading to

embryos of compromised viability (3). To improve conception rates and litter sizes, various hormonal therapies have been employed to enhance luteal development. For example, hCG and GnRH have been administered during the early formation of the corpus luteum (4). Many studies have shown that elevated progesterone (P4) levels promote embryonic development and survival while reducing early embryonic losses in ewes (5). In this context, several investigators have reported that administering hCG or GnRH after natural mating improves conception rates and embryonic growth, and increases litter size in ewes. (6-9).

Furthermore, the administration of these hormones during the luteal phase has been shown to reduce embryonic losses while increasing litter size and lamb birth weight (10).

Evaluation of hormonal treatments administered on day 4 post-mating is therefore essential for understanding their effect on pregnancy loss, conception rate, embryo development, and ultimately, litter size in ewes (11).

The aim of this study was to evaluate the effects of administration human chorionic gonadotropin (hCG), GnRH and progesterone on day 4 after natural mating on reproductive performance and litter size in estrus_synchronized ewes. In addition, the study aimed to determine the correlation between different hormonal treatment and serum progesterone concentration in estrus synchronized ewes.

Materials and methods

Ethical approval

The College of Veterinary Medicine at the University of Mosul provided ethical approval for the research project UM.VET.2025.023 on January 5, 2025.

Estrus synchronization

Thirty-two ewes were synchronized using intravaginal sponges impregnated with 40 mg flugestone acetate for 12 day. At the time of sponge withdrawal (12), each ewe received 625 IU PMSG and was exposed to ten fertile rams. The ewes were randomly assigned to four groups of eight animal each. On day 4 post-mating, the treatments were as follows: Group G1 (Control) received normal saline, Group G2: hCG (500 IU, Pregneal, LG, Korea; i.m.), Group G3: GnRH (0.0042 µg, Gestal, Argentina; i.m.), and Group G4: progesterone (50 mg, i.m.). Following the injection, pregnancy was diagnosed on days 32 and 45 post-mating using a linear rectal ultrasound probe (5–7.5 MHz) to assess embryo viability and detect single or twin pregnancies (13). Blood samples were collected from the jugular vein on days 0, 12, 22, and 32. After centrifugation, serum was stored at –20°C until progesterone analysis by ELISA.

Statistical analysis

Statistical analyses of the data results were calculated using likelihood ratio test of having twin. All results were

analyzed as mean ± standard error (mean ± SE). One-way ANOVA and LSD test estimated the significance between groups, and P values were considered significant at $P \leq 0.05$ (14).

Results

Reproductive performance in terms of estrus response, conception rate, and litter size was improved in ewes that were treated with GnRH, hCG or progesterone on day 4 after natural mating. In the treatment groups, both the estrus response and conception rate reached 100% compared with 90% in the control group, while the likelihood ratio for twin births was 4.58 times greater in the hCG group significantly at $P \leq 0.05$ (Table 1).

Progesterone levels increased in all groups on days 12, 22, and 32 post-synchronization ($P \leq 0.05$, Table 2). Notably, the hCG group exhibited the most pronounced increase in progesterone levels on at days 22 and 32 compared to the other groups, followed by the GnRH group and then the progesterone group ($P \leq 0.05$).

Discussion

The study used hCG therapy on the fourth day following natural mating to improve the conception rate by modifying the formation of an accessory corpus luteum. This modification enlarged the follicle size by approximately 3 mm and stimulated progesterone synthesis, a result that is consistency with (4-11). hCG, which mimics the physiological function of LH, induced oocyte maturation, and prepared the oocyte for ovulation, as well as - facilitated the development of corpus luteum. This was associated with increased luteal tissue weight and an enhanced release of progesterone in ewes (15). Additionally, hCG treatment following natural mating improved conception rates, increased placentation and twinning of lambs, and generally enhanced reproductive performance in ewes at breeding (16). The authors noted the potential role of prostaglandin E2 release in early conceptus development via the EP2 receptor; Progesterone (P4) release is increased when PGE2 activates the corpus luteum's luteinizing hormone receptors. (17).

Table 1: Values of estrus response (%), conception rate and litter size in ewes of study

Groups	No. of ewes	Estrus response (%)	Conception rate (%)	Single-births	twine-births	Litter size- (mean)	Likelihood Ratio- for twins	95% CI Lower, Upper
Control	8	90 %	75%	5	1	0.875	0.55	0.08 - 4.02
hCG	8	100 %	100%	3	5	1.625	4.58 *	1.41- 14.93
GnrH	8	100 %	100%	7	1	1.125	0.39	0.06 - 2.71
Progesterone	8	100%	100%	7	1	1.125	0.39	0.06 - 2.71

* Significant at $P \leq 0.05$: the group is 4.58 times more likely to be associated with twins.

Table 2: Serum progesterone concentration (ng/mL) in ewes following hormonal treatments measured at different time points

Group	Mean \pm Standard Error			
	Day 0 (Pre - synchronization)	Day 12 (post - sponge removal)	Day 22	Day 32
Control	0.41 \pm 0.003 ^{aA}	0.83 \pm 0.003 ^{aB}	0.91 \pm 0.006 ^{aC}	0.96 \pm 0.005 ^{aD}
hCG	0.42 \pm 0.003 ^{aA}	0.84 \pm 0.003 ^{aB}	1.06 \pm 0.005 ^{bC}	1.76 \pm 0.005 ^{bD}
GnrH	0.43 \pm 0.003 ^{aA}	0.84 \pm 0.003 ^{aB}	0.96 \pm 0.006 ^{cC}	1.14 \pm 0.006 ^{cD}
Progesterone	0.42 \pm 0.003 ^{aA}	0.84 \pm 0.003 ^{aB}	0.93 \pm 0.004 ^{dC}	0.99 \pm 0.008 ^{dD}

*Small letters (vertically) indicate significant differences between groups; capital letters (horizontally) indicate significant differences within groups. Values are expressed as mean \pm standard error (ng/mL). Lowercase letters (a-d) within each time point (column) indicate significant differences between treatment groups ($P \leq 0.05$). Uppercase letters (A-D) within each row indicate significant differences over time within the same group ($P \leq 0.05$). Statistical analysis was performed using one-way ANOVA followed by LSD test.

Other investigators have reported an increase in conception rate of 10–20% when hCG or GnRH was given to synchronized ewes during the luteal phase. Furthermore, the development of the embryo and fetal membranes depend on progesterone, which is essential for the regulation of endometrial functions including attachment and placentation in the early luteal phase. An inadequately functioning corpus luteum, producing insufficient progesterone due to a minor LH surge during the per-ovulatory stage, might compromise maturation of follicles as well as the quality of the oocyte, leading to the production low-quality embryos and early embryonic death (18). The injection of GnRH or hCG during the initial luteal stage affects the development of an auxiliary corpus luteum and promotes the release of progesterone into the bloodstream (6,11,19). Researchers have recorded that using hCG or GnRH after the fourth day in estrus-synchronized ewes increases conception rates and twinning rate (7,16,17), and also stimulates early embryo growth (9,19). Moreover, the administration of hCG or GnRH during the luteal phase decreases embryonic losses while increasing litter size and increased birth weight (4,10,20).

The current study indicated that although GnRH administration on the fourth day of synchronization improved estrus response and conception rate, it did not increase litter size because this treatment failed to sufficiently increased serum progesterone (P4) levels. Various researchers have found that an increase in progesterone (P4) enhances early embryonic survival and reduces embryonic loss in sheep (5,21). These findings are consistent with previous studies (10,11,21).

Furthermore, many researchers have provided evidence that GnRH administration after day 4 following estrus synchronization increases progesterone (P4) levels, conception rates, and litter size (8,22,23).

Administration of progesterone increased serum progesterone concentrations; however, the estrous response and conception rate were similar to the control group. Furthermore, treatment with progesterone did not induce the formation of an accessory corpus luteum, as evidenced by similar serum progesterone levels in mated ewes treated with progesterone compared to the control and GnRH groups.

Other authors reported that progesterone analog administration can mimic the formation of the corpus luteum and increase plasma progesterone (24).

In the exogenous progesterone group, serum progesterone concentrations increased in a manner similar to that observed in the other groups. Treatment with progesterone did not result in significant differences in the levels of serum progesterone or litter size compared to the other groups, which is consistent with (15). Progesterone administered during the early embryonic period did not affect conception rate, the pregnancy maintenance, or serum progesterone concentration.

Conclusion

In conclusion, the administration of hCG on day 4 after estrus synchronization effectively stimulated early corpus luteum formation and significantly enhanced reproductive performance in ewes, as evidenced by increased conception rates, larger litter sizes, and higher serum progesterone levels. These findings suggest that post-mating hCG treatment could be a beneficial strategy to improve fertility outcomes in synchronized breeding programs.

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تأثير الهرمون المشيمي البشري والهرمون المحفز للقند والهرمون البروجستيرون على الاداء التناسلي وتراكيز هرمون البروجستيرون في مصل الدم لدى النعاج ذات الشبق الموحد

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الخلاصة

تهدف هذه الدراسة إلى تقييم تأثير إعطاء الهرمون المشيمي البشري (hCG)، والهرمون المحفز للقند التناسلية (GnRH)، والبروجستيرون في اليوم الرابع بعد توحيد الشبق على الحالة التناسلي للنعاج. كما تهدف إلى تحديد العلاقة بين هذه العلاجات الهرمونية وتراكيز البروجستيرون في الدم. تم توحيد الشبق لدى ٣٢ نعجة باستخدام الإسفنجات المهبلية المشبعة، وعند إزالة هذه الإسفنجات، حقنت كل نعجة بـ ٦٢٥ وحدة دولية من هرمون مصل الفرس الحامل (PMSG). ثم قُسمت الحيوانات عشوائياً إلى أربع مجموعات (٨ نعاج لكل مجموعة) المجموعة الأولى: مجموعة السيطرة حقنت بمحلول ملحي. المجموعة الثانية حقنت بـ ٥٠٠ وحدة دولية من هرمون المشيمة البشري hCG المجموعة الثالثة حقنت بـ ٠,٠٠٤٢ ميكروغرام من هرمون المحفز للقند GnRH. المجموعة الرابعة حقنت بـ ٥٠ ملغ من هورمون البروجستيرون. تم تشخيص الحمل باستخدام جهاز الموجات فوق الصوتية عبر المستقيم، كما جُمعت عينات الدم في الأيام ٠، ١٢، ٢٢، و٣٢ لتحليل تركيز البروجستيرون باستخدام تقنية ELISA أظهرت النتائج: تحسّن الأداء التناسلي (الاستجابة للشبق، معدل الحمل، وعدد المواليد) في جميع المجموعات المعالجة مقارنة بالمجموعة السيطرة. بلغت الاستجابة للشبق ومعدلات الحمل ١٠٠% في المجموعات المعالجة، مقارنة بـ ٩٠% في المجموعة السيطرة. كما زادت احتمالية الولادات التوأمية بمقدار ٤,٥٨ مرة في مجموعة hCG. ارتفعت تركيزات البروجستيرون في جميع المجموعات خلال الأيام ١٢، ٢٢، و٣٢، وسجلت أعلى المستويات في مجموعة hCG، تليها GnRH ثم البروجستيرون. الاستنتاج: يحفز إعطاء hCG في اليوم الرابع بعد توحيد الشبق المبكر للجسم الأصفر، مما يحسّن الاداء التناسلي، ويزيد من عدد المواليد ومستويات البروجستيرون في دم النعاج.