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Original Article

Comparison of the effect of Gonadotropin-Releasing Hormone and Human Chorionic Gonadotropin on Reproductive Performance of Romanov Ewes during the Natural Breeding Season

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Abstract

Low pregnancy rate is an important issue in small ruminants. Superovulation protocols would serve as the possible techniques to increase the pregnancy rate in small ruminants due to the use of synthetic hormones. The present study aimed to assess the effect of gonadotropin-releasing hormone (GnRH) and Human chorionic gonadotropin (hCG) after artificial insemination (AI) on the pregnancy rate of Romanov ewes breed. To this end, estrus was synchronized in 140 ewes using a controlled internal drug-releasing (CIDR) device. Following CIDR removal, all ewes received 400 IU Pregnant Mare's Serum Gonadotropin (PMSG). The ewes were assigned to three groups (control, n=20; experimental groups, each of them was divided into three subgroups, n=20). The first group in each experiment was assumed as control. The second and third groups received three levels of GnRH, (400, 500, and 600 µg, n=20) and hCG (200, 300, and 400 IU) administered by the intramuscular (IM) route. As soon as the signs of estrus were observed, the ewes were isolated from the others and artificially inseminated with fresh sperm using the transcervical method. The results indicated that there were no significant differences between experimental groups in ovulation and pregnancy rates on days 33 and 90 post-AI ($P>0.05$). The highest twin birth rate (20%) was recorded in the hCG group (300 IU), as compared to GnRH and control groups ($P<0.05$). The effect of GnRH and hCG on the plasma concentration of progesterone was significant ($P<0.01$). The groups significantly differed in fecundity rate (Single birth, twin birth, and triple birth) ($P<0.05$), all of these parameters were significantly higher in the hCG 300 IU group. It can be concluded that the injection of 300 IU of hCG hormone after insemination improves pregnancy rate in ewes.

Keywords: Artificial insemination, CIDR, GnRH, hCG

1. Introduction

Reproduction is the basis of the survival and generation of all living organisms. In this regard, all new improvements and available facilities need to be used to improve reproductive management in ruminants. Reproductive performance is considered the most important factor in determining the profitability of ruminant breeding. Estrous synchronization and supper ovulation are regarded as valuable management tools used to increase the efficiency of reproduction in small ruminants and seasonal breeder animals due to the use of hormonal therapies (1, 2).

To manipulate animal reproduction, a variety of synthetic hormones are used. These hormones fall into two categories: 1) hormones that stimulate follicular growth, such as follicle-stimulating hormone (FSH), and pregnant mare's serum gonadotropin (PMSG); and 2), as well as those that stimulate the follicle to be released (ovulation), such as gonadotropin hormone-releasing hormone (GnRH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG) (3-5). Gonadotropins that stimulate follicular development are usually administrated when the process of follicular development is restricted in seasonal breeder animals, or when superovulation is needed for multiple embryos recovery for such purposes as embryo banking or embryo transfer (6).

The administration of hCG or other ovulation inducers (e.g., GnRH or LH) to induce accessory corpus luteum (ACL) formation and increase P4 concentration has been investigated in bovine (4) and goats (3, 6) to improve pregnancy rates. The GnRH administration leads to a dramatic increase in progesterone production via the corpus luteum, and consequently, affects fertility rates (7).

Numerous studies support that the increase of P4 improves embryonic survival and reduces embryonic losses in ruminants (8, 9). The previously published studies indicated that the administration of GnRH or hCG after artificial insemination (AI) or natural mating improves pregnancy rate (10). Furthermore, it is of utmost importance to elucidate the possible positive effects of hCG administration on different ewes breeds under different climate conditions and management systems.

In light of the aforementioned study, the present study aimed to determine the effect of different doses of GnRH and hCG 12 days after artificial insemination on pregnancy rate, pregnancy loss, rate of multiple births number of lambs and lambing rate, number of Graafian follicles, and P4 concentration in Romanov sheep in the non-breeding season in Southern Khorasan Province, Iran.

2. Materials and Methods

2.1. Animals, Experimental Diets, and Management

This study was conducted in the Research Unit Farm of the Light Livestock Breeding Center of the Water and Soil Conservation Company, located in the southern Khorasan province, Iran (Longitude 449 03. 34 Latitude 727 25 25 5). A total of 140 Romanov ewes (within the age range of 2-3 years and a mean bodyweight of 52.5±4.5 kg) were included in the experiment. The estrous cycle of ewes in the non-breeding season was synchronized with a controlled internal drug-releasing (CIDR)device (Intervet, Holand) for 14 days. Following CIDR removal, all ewes received 400 IU PMSG. The animals were maintained under natural grazing and had free access to water.

After removing the CIDR, to evaluate the effects of GnRH and hCG with different doses, all ewes were assigned to three groups (control, n=20; treatments: hCG n=60, and GnRH, n=60). Treatment groups received GnRH and hCG intramuscularly at three levels, 400 (n=20), 500 (n=20), and 600 µg (n=20), as well as 200, 300 and 400 IU (n=20), respectively. As soon as the signs of estrus were observed, they were isolated from the other ewes, and artificial insemination was performed with fresh sperm using the transcervical method.

2.2. Reproductive Performance and P4 and Glucose Concentration Determinations

Ultrasonography examination was carried out by an ultrasound device (6 MHz linear probe, ECM model, France). The number of Graafian follicles (follicles larger than 4 mm) was simultaneously examined with CIDR removal and estrus observation. The corpus luteum was assessed 10 days after CIDR removal. Pregnancy rates were assessed by the same transrectal ultrasonography equipment and the same technician 34 days after artificial insemination. Thereafter, in each experiment parameters, such as the time of estrus initiation (hour), rate of return to estrus, parturition rate, rate of multiple births, number of lambs, and rate of lambing were evaluated. Blood samples (5 ml) were collected 14 days after artificial insemination by the Venoject tube. Plasma P4 concentration (ng/mL) was determined by radioimmunoassay (RIA) using commercial RIA kits (Beckman Coulter; Immunotech, Marseille, France).

2.3. Statistical Analysis

The number of CL and plasma P4 concentrations were analyzed for the main effect of treatment and days of the estrous cycle (within each group) using one-way ANOVA. Differences between means were determined by Tukey's test. A p-value less than 0.05 was considered statistically significant. The results were reported as mean±SEM. All statistical analyses were performed using the System for Statistical Analysis (SAEG) software (Ribeiro Junior, 2001).

3. Results

3.1. Estrus Rate and the Average Time Interval between CIDR Removals up to Estrus

The results of estrus rate and the average time interval between CIDR removal up to estrus in different experimental groups, as well as the number of Graafian follicles and corpus luteum at the time of estrus, and CIDR removal are presented in table 1. There were no significant differences between experimental groups in estrus rate and number of CLs, 14 days after AI in different groups (*P*>0.05). Consequently, the number of follicles and CLs on the day of CIDR removal and 14 days after AI were higher in the hCG group, as compared to GnRH and control groups (*P*<0.05).

3.2. Reproductive Performance

The results of reproductive performance in the GnRH, hCG, and control groups are illustrated in table 2. There were no significant differences between experimental groups in ovulation and pregnancy rates on days 33 and 90 post-AI (*P*>0.05). Significant differences were recorded in the rate of fecundity (Single birth, twin birth, and triple birth) between different groups (*P*<0.05). The highest and lowest rates of single birth were obtained at (60%) and (10%) in the control and hCG groups (300 IU), respectively (*P*<0.05). Moreover, the highest and lowest rates of twin birth were reported as 70% and 35% in the hCG (300 IU) and control groups, respectively (*P*<0.05). The hCG group (300 IU) showed the highest twin birth rate (20%). This rate in the 300 IU hCG group significantly differed, as compared to GnRH and control groups (*P*<0.05). There were no differences between experimental groups in Lamb loss rate, maternity and male rate, and survival rate (*P*>0.05). Nonetheless, there was a slight increase in the proportion of female birth rates.

3.3. Concentrations of P4 and Glucose

The results of the mean concentrations of P4 in the GnRH, hCG, and control groups are presented in table 3. The mean concentrations of P4 were higher in the hCG groups (9.27, 9.81, 9.85 ng/mL), compared to the GnRH (8.19, 8.41, 8.51 ng/ml) and control groups (8.21 ng/ml) (*P*<0.05; Table 3).

Table 1. Estrus rate and the average time interval between CIDR removal up to estrus

Reproductive parameters	Experimental Group							
	Control n=20	GnRH n=60			hCG n=60			*P value*
	PMSG n=20	400 µg n=20	500 µg n=20	600 µg n=20	200 IU n=20	300 IU n=20	400 IU n=20	
Estrus rate (%)	100	100	100	100	100	100	100	
CIDR Removal up to estrus (hour)	36.21	35.19	33.51	34.41	32.22	32.22	33.33	0.012
Follicles on estrus (follicles/ewes)	(86/20) 4.30^{c}	(95/20) 4.75^{a}	(90/20) 4.50^{bc}	(88/20) 4.40^{bc}	(98/20) 4.90^{a}	(96/20) 4.80^{a}	(97/20) 4.85^{a}	0.004
The number of follicles on CIDR removal day (follicles/ewes)	(42/20) 2.10^{c}	(50/20) 2.50^{b}	(46/20) 2.30^{bc}	(47/20) 2.35^{bc}	(52/20) 2.60^{a}	(56/20) 2.80^{a}	(54/20) 2.70^{a}	0.007
Number of CL 14 days post AI (CL/ewes)	(42/20) 2.10^{c}	(45/20) 2.25^{b}	(44/20) 2.20^{b}	(44/20) 2.20^{b}	(51/20) 2.55^{a}	(55/20) 2.75^{a}	(50/20) 2.50^{a}	0.001

[a, b,c]: Means within the same row with different superscripts differ significantly (*P*<0.05)

Table 2. Reproductive performance of Romanov ewes

Reproductive parameters	Experimental Group							
	Control n=20	GnRH n=60			hCG n=60			*P value*
	PMSG n=20	400 µg n=20	500 µg n=20	600 µg n=20	200 IU n=20	300 IU n=20	400 IU n=20	
Ovulation rate (follicles/ewes) (%)	(19/20) 95	(20/20) 100	(19/20) 95	(19/20) 95	(15/20) 100	(20/20) 100	(20/20) 100	0.50
Pregnancy rate at Day 33 (%)	(15/20) 75	(15/20) 75	(14/20) 70	(15/20) 75	(15/20) 75	(16/20) 80	(15/20) 75	0.32
Pregnancy rate at Day 90 (%)	(15/20) 75	(15/20) 75	(14/20) 70	(15/20) 75	(15/20) 75	(15/20) 75	(15/20) 75	0.31
Single birth (%)	(8/20) 40^{a}	(6/20) 30^{a}	(5/20) 25^{ab}	(6/20) 30^{a}	(1/20) 5^{b}	(2/20) 10^{bc}	(3/20) 15^{a}	0.06
Twin births %)	(7/20) 35^{cb}	(9/20) 45^{b}	(9/20) 45^{a}	(9/20) 45^{ab}	(12/20) 60^{a}	(12/20) 60^{a}	(10/20) 50^{a}	0.07
Triplet births (%)	(0/20) 0^{b}	(0/20) 0^{b}	(0/20) 0^{b}	(0/20) 0^{b}	(2/20) 10^{a}	(1/20) 5^{a}	(2/20) 10^{a}	0.06
Fecundity (%)	(21/20) 1.05^{b}	(24/20) 1.2^{b}	(23/20) 1.15^{b}	(24/20) 1.2^{b}	(31/20) 1.55^{a}	(29/20) 1.45^{a}	(29/20) 1.45^{a}	0.06
Lamb loss rate (%)	(0/21) 0	(0/24) 0	(2/23) 10	(3/24) 12	(0/31) 0	(0/29) 0	(0/29) 0	0.52
Maternity rate (%)	(15/21) 71	(13/24) 54	(14/23) 60	(13/24) 54	(15/31) 48	(15/29) 51	(13/29) 44	0.56
Male rate (%)	(6/21) 29	(11/24) 46	(11/23) 40	(11/24) 46	(16/31) 52	(14/29) 49	(16/29) 56	0.53
Survival rate (%)	(21/21) 100	(24/24) 100	(20/23) 87	(21/24) 87	(31/31) 100	(29/29) 100	(29/29) 100	0.27

[a, b,c]: Means within the same row with different superscripts differ significantly (P<0.05)

Table 3. Mean concentrations of P4

Reproductive parameters	Experimental Group							
	Control n=20	GnRH n=60			hCG n=60			*P value*
	n=20	400 µg n=20	500 µg n=20	600 µg n=20	200 IU n=20	300 IU n=20	400 IU n=20	
P4 (ng/ml)	8.21^{b}	8.19^{b}	8.51^{b}	8.41^{b}	9.27^{a}	9.81^{a}	9.85^{a}	0.001

[a, b,c]: Mean concentrations of P4 and Glucose in the GnRH, hCG, and Control groups

4. Discussion

There were no differences between the experimental groups in terms of observation of estrus symptoms. The average time interval between CIDR removals up to estrus in experimental groups was in the range of 30-40 h. The results of some previous studies have demonstrated that the use of CIDR with low doses of eCG has a scant effect on follicle growth and ovulation, especially outside the reproductive season (11). The results of the present study pointed to the positive role of hCG in a marked improvement in reproductive function, the growth and development of follicles, as well as the number of corpus luteum.

Ovulation in most ewes, depending on the breed, occurs at a fixed time in relation to the onset of estrus (12). The breed of ewe could be one of the factors influencing the time of ovulation. The results of this study supported the hypothesis that GnRH or hCG treatment improves pregnancy rate as soon as the emergence of the signs of estrus following insemination. On the contrary, Fukui, Itagaki (4) reported that the administration of hCG on day 4 after insemination did not improve fertility. Other authors observed similar results after the application of GnRH, hCG, or eCG at the mid-luteal phase (13, 14).

In agreement with this finding, other researchers observed an increase in pregnancy rate by 10% and 20% when ewes were treated with hCG or GnRH at mating or in the luteal phase (7, 11, or 13 days post-mating or AI) (10, 15, 16). This discrepancy in the reported results could be attributed to different protocols, management systems, nutritional status, or physiological status resulting from diverse experimental conditions. Furthermore, these inconsistent results may indicate that breed, timing of treatment, or doses used in these treatments exert critical effects on fertility rate and embryo survival.

The results of the present study pointed out that the use of hCG or gonadotropin-releasing hormone in ewes under the program of induction of multiple ovulation with gonadotropins in equestrian couples had a double rate, compared to the group using only equine gonadotropins (17). The injection of GnRH, or its analog, encourages a sudden increase in LH and FSH, and 2 h after the injection, all ewes will demonstrate a sudden increase in LH. A group of researchers has reported that the use of GnRH on the day of inoculation increases the weight of the corpus luteum and helps increase the secretion of progesterone from the auxiliary corpus luteum by helping the growth of small yellow objects (18).

Another group of researchers indicated that the injection of GnRH during estrus may affect ovulation time, fertility rate, corpus luteum development, progesterone secretion, and embryonic survival. Recently, the use of gonadotropins 12 days after sheep inoculation has been reported to improve the rate of pregnancy, twinning, and lamb birth by increasing fetal survival (11).

In a study by Cam, Kuran (19) conducted on cattle, the use of gonadotropins on the 1st and 12th days after inoculation increased fetal survival, pregnancy rate, and birth rate. Using GnRH on the 10th, 11th, 12^{th}, and 13^{th} days of pregnancy can improve fetal survival and pregnancy rates. Moreover, it increases blood progesterone levels, which in turn, helps increase progesterone due to the placement of auxiliary corpus luteum. Day 12 is a critical time for mothers to detect pregnancy (20). If the progesterone level is insufficient, the embryo will not be able to form a placenta and will be lost since it is the time when the corpus luteum recedes in the natural cycle of the estrous cycle (20).

In line with the results of the study by Mee, Stevenson (21), the findings of the present research indicated that the use of Using GnRH can affect the fertility rate by increasing the production of progesterone by the corpus luteum. Nonetheless, Lucy and Stevenson (7) reported inconsistent results. The findings of the present study also were in agreement with those reported by Cam, Kuran (19) who demonstrated that the use of GnRH on the 12th day after inoculation improves the reproductive function of ewes. In some studies, it has been reported that eCG injection before or at the time of removing the sponge containing progesterone led to an improvement in ovulation, twin birth rate, and lamination in different breeds of sheep during the breeding and non-breeding seasons (22).

Nevertheless, contradictory results were reported in some other studies. It has been indicated that the use of gonadotropins in equine can have a negative effect on the rate of pregnancy. In a similar vein, this effect is also expected and recorded in the reproductive function of ewes (23). The long-lasting half-life of hCG appears to cause a large number of ovarian-resistant follicles on the surface of the ovary, which in turn leads to increased estrogen levels. High levels of estrogen also affect various reproductive activities, including the rate of multiple ovulation, sperm transfer, fetal fertility, and survival (22). One effective way to prevent the negative effects of eCG is to substitute it with hCG or GnRH in animals subjected to the supper ovulation procedure. Contradictory results have been reported for the use of GnRH and hCG in different studies.

Progesterone is the most important factor in the survival of the corpus luteum, the presence of which is essential for the continuation of pregnancy (24). The use of GnRH and hCG lead to a dramatic increase in LH secretion. This increment in the LH secretion, along with the fact that the small luteal cells of the corpus luteum contain more LH receptors, consequently resulted in the secretion of more amount of progesterone. Based on the aforementioned issues, it can be stated that one of the reasons for the successful pregnancy of ewes is the high level of progesterone in their blood serum (18).

The results of the current study pointed out that the administration of hCG on day 14 after artificial insemination consistently brought about a dramatic increase in plasma concentration of P4. A series of studies demonstrated the positive relationship of early and mid-luteal phase concentrations of progesterone with subsequent embryo survival rate reviewed by Diskin and Morris (25). In addition, researchers have suggested that when the estrous cycle was induced in ewes, plasma P4 concentration was 70% lower than that in animals with natural estrus and breeding cycles (26). A study conducted by Kittok, Stellflug (16) reported that the administration of hCG on Days 11, 12, and 13 after mating resulted in significantly higher pregnancy rates in treated ewes (58%), as compared to those in untreated ewes (29%).

5. Conclusion

The administration of hCG or GnRH on Day 14 post artificial insemination performance reproductive. Furthermore, the reproductive efficiency after hormonal treatments was affected by external factors, such as the fertility of rams used in the fixed time artificial insemination (FTAI) or the body condition of the ewes at FTAI. Treatment with hCG in inseminated ewes increased the percentage of pregnancy.

Authors' Contribution

It is worth noting that M. D. and M. V. carried out the experiment. M. D. wrote the manuscript with support from M. V., M. D. conceived the original idea and supervised the project.

Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed the ethical committee of the University of Birjand, Birjand, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest.

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