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EFFECT OF NUMBER AND SPERM CONCENTRATION OF ARTIFICIAL INSEMINATIONS ON THE REPRODUCTIVE PERFORMANCE OF GnRH-PGF$_2\alpha$-GnRH TREATED CROSS-BREED EWES IN THE BREEDING SEASON.

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ABSTRACT

The objectives of this study was to evaluate the effects of GnRH- PGF$_2\alpha$–GnRH protocols (on 0, 5 and 7 d, respectively) and artificial insemination (AI) within 24-28 h post the 2nd GnRH injection, on reproductive performance of crossbred ewes, in reference with comparing the same AI dose once versus twice or once AI with low versus high concentration. Total of 40 ewes 1/2 Finnish Landrance x 1/2 Rahmani (F x R) and 1/2 Finnish Landrance x 1/2 Ossimi ewes (F x O) during January breeding season 2012 were used in this study. Animals were divided into four experimental groups (10 in each, 5 from each crossbred). In the 1st group, ewes were exposed to fertile ram without treatment (G1, control). Ewes in treatment groups were i.m. injected (d 0) with 1 ml GnRH analogue (Receptal), followed 5 d later by i.m. injection with 0.7 ml PGF$_2\alpha$ (Estrumate). A second dose of 1 ml GnRH was given on d 7 and AI was carried out after the 2nd GnRH injection based on the experimental design. Ewes in the treatment groups received the same hormonal protocol, but differed in sperm concentration of AI dose, being about 300 x 10^6 /ml/once after 24 h from the 2nd GnRH injection (G2), 300 x 10^6/ml/twice after 20 and 28 h from the 2nd GnRH injection (G3) and 400 x 10^6/ml/once after 24 h from the 2nd GnRH injection (G4). Results revealed that estrus rate was the highest (70%) in G2, moderate in G3 (60%) and the lowest in G4 (50%) (P<0.05), being higher (P<0.05) in F x O than in F x R within each group. No pronounced differences were detected among groups or crosses in the time of incidence and duration of estrus. Lambing rate based on treated ewes decreased (P≥0.05) by increasing number of AI (once vs. twice) from 50% in G2 to 40% in G3, however, it decreased (P<0.05) from 40 to 20% by increasing AI dose from 300 to 400 x 10^6 sperm/ml/once AI in G3 and G4, respectively. Lambing rate based on estral ewes increased (P<0.05) by increasing AI number (50 vs. 66.7%) in G2 and G3, respectively; while it decreased (P≥0.05) from 50 to 40% by increasing AI dose in G3 and G4, respectively. There was higher lambing rate of F x R than that of F x O in G2 and G4 and G1. All treatments shortened lambing period as compared to the control group, ranging between 1-5 d in treatment groups and averaged 33 d in the control one. Lambs were numerous in G2 as compared to G1 (7 vs. 6 lambs). Litter size was higher (P<0.05) in G2 than in G1 and lower in G3 (1.25) than in G4 (1.50, P<0.05). Litter size was higher (P<0.05) in F x R than in F x O. Ovarian activity was higher in G2 and G3 than in G4 as well as in F x R than in F x O ewes. On day of 1st GnRH injection, P4 concentration was elevated in all groups, increased post-1st GnRH injection, decreased post- PGF2α injection and at AI and showed marked increase 5 days post-AI. Loss cases was higher (P<0.05) in G3 than in G4 (37.5 vs. 25%), moderate in G2 (30%). Overall rate of loss was 31.8% in all treatment groups.

In conclusion, using GnRH- PGF$_2\alpha$–GnRH protocols (on 0, 5 and 7 d, respectively) and AI 24 h post the 2nd GnRH injection during breeding season give high sufficient of estrous synchronization and lambing as well as the lowest reproductive losses , in particular for F x R ewes.

Keywords: Ewe, GnRH, fertility, progesterone, embryonic loss.

INTRODUCTION

Estrus synchronization of ewes has been accomplished using several methods with various degrees of success. Progesterone (P4) impregnated intravaginal sponges, left *in situ* for 12-17 days in the breeding season, is a widely used method for estrus synchronization of ewes. Another equally effective method is two injections of PGF2α at 11-day interval. Although the two methods used during breeding season can give high/sufficient rates of synchronization and lambing, they have some disadvantages of being applied for a long period (Larsson *et al.*, 1991).

In the cyclic animals, a follicular wave terminates when the dominant follicle either regresses or ovulates, leading to the start of a new wave of follicular growth. An injection of GnRH analogues 6 days prior to an injection of PGF2α, enhances conception rate (Stevenson *et al.*, 1996), increases number of synchronized animals, and reduces variability of time to estrus (Twagiramungu *et al.*, 1992). This decrease may be explained by the initiation of a new follicular wave following injection of GnRH, which results in a new dominant follicle, being present at the time of PGF2α injection (Pursley *et al.*, 1998).

Embryonic and fetal mortalities (EFM) are the most important causes of reproductive losses and have a great effect on the fertility of domestic animals (Vanroose *et al.*, 2000). EFM has been estimated to be about 30 % in sheep and goats (Chaudhary and Purohit, 2012). The impact of economic losses resulting from fetal death include not only the loss of offspring or decreasing the crop yield of calves and kids, but also a prolonged “open” period for the dam leading to increased culling rates (Jonker 2004). Causes of EFM can be divided into non- infectious and infectious categories (Christianson, 1992). There are many tools for diagnosis of embryonic/fetal losses. Real time B-mode ultrasonography provides a simple, rapid, accurate and non-invasive means for pregnancy diagnosis and counting fetal numbers as well as considered a good alternative method to check embryonic mortality in living in small ruminants on the farm (Dixon *et al.*, 2007). Few studies were undertaken to investigate EFM in goats (Engeland *et al.*, 1999 and Zamfirescu *et al.*, 2011). Moreover, most previous studies in sheep and goats were carried out in a certain geographical location and focused on a single ultrasonographic scanning during gestation and comparing it to the birth records (Dixon, 2003; Zamfirescu *et al.*, 2011). Nevertheless, no previous studies have been done to monitor the incidences of EFM during different stages of gestation in sheep.

The objectives of this study was to evaluate the effects of GnRH-$PGF_2\alpha$–GnRH protocols (on 0, 5 and 7 d, respectively) and artificial insemination (AI) within 24-28 h post the 2^{nd} GnRH injection, on reproductive performance of crossbred ewes (Finland x Rahmani and Finland x Ossimi), in reference with comparing the same AI dose once versus twice or once AI with low versus high dose.

MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, belonging to Animal Production Research Institute, Ministry of Agriculture, in cooperation with Department of Animal Production, Faculty of Agriculture, Mansoura University, during January, 2012 breeding season.

Animals, housing and feeding system:

Total of 40 ewes 1/2 Finnish Landrance x 1/2 Rahmani (F x R) and 1/2 Finnish Landrance x 1/2 Ossimi (F x O) ewes during January breeding season 2012 were used in this study. Animals were divided into four experimental groups (10 in each, 5 from each crossbred).

Animals were housed in semi-open sheds in groups. They were fed concentrate feed mixture and roughages according to NRC (2001). The daily feed offered per ewe composed of 1.250 kg concentrate feed mixture (14% CP) and 5 kg Egyptian clover with free access to trace mineralized salt lick blocks and drinking water all time.

Experimental design:

In the 1st group, ewes were exposed to fertile ram from the contemporary to that of treatment ewes start time up to the end of the breeding season or for a period covering 2 estrous cycles. During breeding season, ram of proven fertility was introduced to ewes for 35 days and was rested for 7 days and again introduced for another period of 35 days or until the end of the experiment (G1, control)**.**

However, ewes in treatment groups (G2, G3 and G4), were i.m. injected (day 0) with 1 ml GnRH analogue (Receptal, Intervet International B.V. Boxmeer-Holland, Each ml of Receptal contained 0.0042 mg Buserelin acetate equivalent to 0.004 mg Buserelin), followed 5 days later by i.m. injection with 0.7 ml $PGF_2\alpha$ (Estrumate, Coopers Animal Health LTD, Berkhamsted-England, Each ml of Estrumate contained 263 μg Cloprostenol Sodium equivalent to 250 μg Cloprostenol.

A second dose of 1 ml GnRH analogue was given on day 7 and artificial insemination of treated does was carried out after the 2nd GnRH injection based on the experimental design.

Ewes in the treatment groups received the same hormonal protocol, but differed in sperm concentration of AI dose, being about 300 x 10^6 /ml/once after 24 h from the 2nd GnRH injection (G2), 300 x 10^6/ml/twice after 20 and 28 h from the 2nd GnRH injection (G3) and 400 x 10^6/ml/once after 24 h from the 2nd GnRH injection (G4).

Artificial insemination:

Fresh diluted semen was used. Semen was collected by the use of artificial vagina and aid of natural estral ewes. Semen was diluted just before insemination. Immediately after semen collection the volume was measured and samples of raw semen were taken for determination of sperm motility according to the method described by Bane (1982). Only ejaculates of >80% initial motility were diluted.

The Tris-yolk extender was used for the extension of fresh semen. The extender was prepared and kept at 5°C for 24 h before semen dilution.

For fresh semen, each 100 ml of Tris-yolk extender consisted of 3.025 g Tris, 1.675 g citric acid, 0.75 g glucose, 15 ml egg yolk, 1 ml antibiotics including 100.000 IU penicillin and 100.000 µg streptomycin and distilled water up to 100 ml (Leboeuf *et al.*, 2000). All chemicals used for preparation of extender were purchased from Sigma Chemical Company (P.O. Box 14508, Sant Louis, MO 63178, USA). The extender was gently shacked and warmed up to 37^{o}C using a water bath before semen dilution. The collected semen was diluted (1 part semen: 4 parts extender) at 37^{o}C and sperm motility remaining over 70%. Sperm concentration was adjusted according to the experimental design. Insemination was carried out using a simple inseminating pipette with fine blunt bent end and a vaginal speculum (Plate 1). Semen was deposited into the cervix as far as possible (about 1 cm).

Reproductive efficiency:

Many parameters are used to determine reproductive efficiency according to Jainudeen and Hafez (1993). These parameters included estrus rate, onset and duration of estrus, lambing rate, number of lamb borns, litter size as well as type and sex of births.

Blood sampling:

Blood samples were taken morning before feeding via the jugular vein from all ewes into evacuated tubes (10 ml). Just after sampling, the blood samples were separated to obtain serum by centrifugation of blood at 2500 rpm for 15 min. Serum was packed in labeled plastic tubes and stored at -20^{o}C until assayed later for progesterone concentration. Blood sampling started -1, 0, 5, 7, 8 and 13 days from the start of the experiment.

Ultrasonography examination:

Ewes were not withheld from food and water before ultrasonography examination. During the examination each ewe was placed in dorsal recumbence. A portable Scanner (480 Vet, Pie Medical, Maastricht, Netherlands) with a 5 MHz rectal transducer was used. The probe was fitted into rectal rod (3 x 64 cm) and contact gel was applied to the surface of the transducer and rod to provide better contact and lubrication. The rod was inserted into the rectum, 15 or 20 cm deep, to be able to scan the uterine horns. The surface of the transducer was first towards the right ileum to scan the bladder and then it was rotated 120° to 180° clockwise or counterclockwise across the uterine horns to scan the entire pelvic region in each group. Ewes were diagnosed using ultrasonography on day of injections, AI and 5 d post-AI.

Progesterone assay:

Progesterone concentration was determined by Radioimmunoassay procedure in samples of selected 5 animals (3 does kidded + 2 does of which did not kid) of each treatment group in each experiment.

Quantitative determination of progesterone in serum samples was carried out using progesterone radioimmunoassay kit (catalog No. 1188 manufactured by Immunotech, France). The assay is based on competition reaction (Bojanic *et al.*, 1991). Samples reaction (50 µl for progesterone) were incubated 1 h with I^{125} labeled progesterone (500 µl), as tracer, in antibody-coated tube. After incubation the liquid contents of the tubes are aspirated and bound radioactivity is measured to determine progesterone in

serum using automatic Mini-Gama counter (LKB 1275, USA). The sensitivity of the assay progesterone was 0.03 ng/ml. While, coefficient of variation 4.3% for both progesterone intra- and inter-assay, respectively.

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1982) using computer program of SAS system (2000).

The obtained percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages. The significant differences were carried out using Multiple Range Test of Dancan (1955).

RESULTS AND DISCUSSION

Estrous activity post-$PGF_2\alpha$ injection of treatment groups:

Results presented in Table 1 revealed that ewes in all treatment groups showed estrous activity at different rates within 36-72 h post-$PGF_2\alpha$ injection. Estrus rate was significantly ($P<0.05$) different among treatment groups, being the highest (70%) in G2, moderate in G3 (60%) and the lowest in G4 (50%). The observed group differences were attributed to the variation in response of treated ewes to $PGF_2\alpha$ injection, because ewes in all groups received the same treatments. On the other hand, there was markedly significant differences in estrus rate between crosses, being significantly ($P<0.05$) higher in F x O than in F x R within each group.

However, no pronounced differences were detected among groups or crosses in the time of incidence estrous and even in estrous duration, ranging 41.7-42.6 h and 32.7-33.1 h, respectively. It is worthy noting that ewes in the control group (G1) did not include for observation of estrous activity. Similar results were reported by Abdel-Salam (2010) on ewes treated with the same protocol used in this study.

The obtained results regarding the recorded estrous rates of treated ewe indicated group variation beside cross-bred differences in response to $PGF_2\alpha$ injection. In accordance with the present results, Ataman and Aköz (2006) found that GnRH-PGF2α technique allowed to induce the estrous response in 93.3 and 86.6% in the GnRH-PGF2α- and double PGF2α-treated ewes (received 10 µg of busereline and 5 d later injection of 0.294 mg of Triaprost tromethamine, an analogue of PGF2α). In comparable with the present results, higher estrous rates (90 and 100%) were reported by Beck *et al.* (1996) and Öztürkler *et al.* (2003) in ewes treated with double PGF2α.

Table (1): Estrus rate (%), time of incidence (h) and duration (h) of estrus after $PGF_2\alpha$ injection for cross-bred ewes in treatment groups.

Experimental group		N	Estrus response to post-$PGF_2\alpha$ treatment			
			Ewes in estrus		Estrous activity	
			n	%	Incidence* (h)	Duration (h)
G2	F x R	5	3	60^{b}	41.6±1.52	33.0±1.3
	F x O	5	4	80^{a}	41.8±1.04	32.8±1.4
Mean		10	7	70^{A}	41.7±1.28	32.9±1.2
G3	F x R	5	2	40^{b}	42.4±1.60	33.7±1.6
	F x O	5	4	80^{a}	42.6±1.75	32.6±1.4
Mean		10	6	60^{AB}	42.3±1.67	33.1±1.5
G4	F x R	5	2	40^{b}	42.7±1.33	32.6±1.7
	F x O	5	3	60^{a}	42.5±1.74	32.8±2.0
Mean		10	4	50^{B}	42.6±1.53	32.7±1.8

G2: $300x10^6$ sperm/once. G3: $300x10^6$ twice. G4: 400 $x10^6$ sperm/once.
Means within the same column with different superscripts (small or capital litters) are significantly different at P<0.05. N: Number of treated ewes.

Lambing performance of ewes in the experimental groups:

Data shown in Table 2 cleared significant (P<0.05) effect of treatment and cross-bred on lambing rate of ewes recorded based on ewes showing estrous activity or total number of treated ewes. The observed group differences were related to number and/or dose of AI. Results showed that lambing rate based on total number of treated ewes insignificantly decreased by increasing number of AI (once vs. twice) from 50 in G2 to 40% in G3, however, it decreased significantly (P<0.05) from 40 to 20% by increasing AI dose from 300 vs. 400 x 10^6 sperm/ml/once AI in G3 and G4, respectively. It is worthy noting that lambing rate based on number of estral ewes significantly (P<0.05) increased by increasing AI number (50 vs. 66.7%) in G2 and G3, respectively; while it insignificantly decreased from 50 to 40% by increasing AI dose in G3 and G4, respectively. In contrast to the present results of lambing rate in G2 vs. G4 (300 vs. 400 x 10^6 sperm/ml), Ashmawy *et al.* (2012) found that fertility rate of ewes was 50% due to high insemination dose (300 x 10^6 motile sperm), 42.10% for the medium (200 x 106 motile sperm) and 31.57% for the low one (100 x 10^6 motile sperm). Difference was only significant (P<0.05) between high and low insemination dose.

In comparing treatment groups with the control one, it was found that lambing rate was significantly (P<0.05) lower in G4 than in the control (G1) based on total number of treated ewes (50 vs. 20%) and than in all treatment groups based on estral ewes (83.3 vs. 40-66.7%). Results of lambing rate obtained in this study are seemingly not reasonable as cervical insemination in sheep resulted in lambing rates of 40% (Halbert *et al.*, 1990). Also, Sallam (1999) concluded fertility rate in sheep around this figure resulted from research study.

Table (2): Lambing characteristics of ewes in different experimental groups.

Exp. group		N[1]	Mated ewes		Lambed ewes			Lambing period	
			n[2]	%	n[3]	%[3/1]	%[3/2]	Date	Days
G1	F x R	5	3	60	3	60^{a}	100.0^{a}	1/6/2012-30/6/2012	30
	F x O	5	3	60	2	40^{b}	66.7^{b}	2/6/2012-03/7/2012	31
Mean		10	6	60^{AB}	5	50^{A}	83.3^{A}	1/6/2012-03/7/2012	33
G2	F x R	5	3	60^{b}	3	60^{a}	100^{a}	17/6/2012-20/6/2012	4
	F x O	5	4	80^{a}	2	40^{b}	50.0^{b}	16/6/2012-18/6/2012	3
Mean		10	7	70^{A}	5	50^{A}	50^{C}	16/6/2012-20/6/2012	5
G3	F x R	5	2	40^{b}	1	20^{b}	50^{b}	17/6/2012 ----------	1
	F x O	5	4	80^{a}	3	60^{a}	75^{a}	16/6/2012-18/6/2012	3
Mean		10	6	60^{AB}	4	40^{A}	66.7^{B}	16/6/2012-18/6/2012	3
G4	F x R	5	2	40^{b}	1	20	50.0^{a}	16/6/2012----------	1
	F x O	5	3	60^{a}	1	20	33.3^{b}	16/6/2012 ----------	1
Mean		10	5	50^{B}	2	20^{B}	40.0^{C}	16/6/2012 ----------	1

G1 : control. G2: 300x10^{6} sperm/once. G3: 300x10^{6} twice/twice. G4: 400 x10^{6} sperm/once. Means within the same column with different superscripts (small or capital litters) are significantly different at P<0.05. N[1]: Number of treated ewes.

In the present work, the high inseminate (300 x 10^{6} sperm) resulted in higher lambing rate than (400 x 10^{6} sperm), which could be recommended only in case of availability of semen (Ashmawy *et al.*, 2012). Therefore, such results are not expected and no reasons for these trends of lambing rate in different treatment groups.

The effect of interaction between cross-bred and treatment on lambing rate was significant, reflecting higher lambing rate of F x R than that of F x O in G2 and G4 treatment groups and control one (G1), except that in G3 (twice AI), which showed an opposite trend (Table 2).

Remarkable effect of treatment was recorded on lambing period of ewes (Table 2). Results showed that all treatments shortened this period as compared to the control group during January breeding season, ranging between 1-5 d in treatment groups and averaged 33 d in the control one.

Litter size and yield of lambs in the experimental groups:

Results shown in Table 3 revealed that inspite an equal number of lambed ewes in G2 and G1, lamb borns were numerous in G2 as compared to G1 (7 vs. 6 lambs). This reflected in significantly (P<0.05) higher litter size in G2 than in G1. Although the number of lambed ewes and lamb borns were lower in G3 and G4 than in G1, litter size was higher in G3 (1.25 lambs/ewe, P≤0.05) and G4 (1.50 lambs/ewe, P<0.05) than in G1 (1.20 lambs/ewe). Generally, litter size was higher in F x R than in F x O cross-bred.

Table (3): Number, sex and type of births (lambs), and litter size of ewes in different experimental groups.

Group		N	Lambed ewes	Number of lambs	Litter size per ewe	Sex of lambs		Type of birth	
						♀	♂	Twins	Single
G1	F x R	5	3	4	1.33^{a}	2	2	1	2
	F x O	5	2	2	1.00^{b}	1	1	-	2
Mean		10	5	6	1.20^{C}	3	3	1	4
G2	F x R	5	3	5	1.67^{a}	3	2	1	3
	F x O	5	2	2	1.00^{b}	1	1	-	2
Mean		10	5	7	1.40^{AB}	4	3	1	5
G3	F x R	5	1	2	2.00^{a}	1	1	1	-
	F x O	5	3	3	1.00^{b}	2	1	1	1
Mean		10	4	5	1.25^{BC}	3	2	2	1
G4	F x R	5	1	2	2.00^{a}	1	1	-	2
	F x O	5	1	1	1.00^{b}	1	-	-	1
Mean		10	2	3	1.50^{A}	2	1	-	3

Means within the same column with different superscripts are significantly different at 5% level. a and b: crossbred differences. A, B and C: group differences. N^{1}: Number of treated ewes

In addition, sex of lambs tended to be slightly more female than male lambs in all groups. However, type of births was almost in single borns in treatment and control groups, except for that in G3, there was a tendency of increasing twins production. Such trend in G3 may be associated with increasing number of AI (once vs. twice AI). No pronounced effect was found for cross-bred ewes on sex of lmbs or type of births (Table 3).

Ovarian activity of treatment groups:

Results of ultrasonographic examination presented in Table 4 revealed that number of corpus luteum (CL) and follicles increased post-1st GnRH injection. This finding is based on that synchronization of follicular growth of ewes is through ovulation of the dominant follicle of the present follicular wave at the injection of 1st GnRH injection and initiation of new follicular growth wave (Gordon, 1996). Also, the initial GnRH treatment should induce a sufficient release of follicle stimulating hormone (FSH), and luteinizing hormone (LH) (acute phase flare effect) and then cause ovulation or luteinization of the dominant follicle (Glazar *et al.*, 2004). This finding may indicate that all treated groups following GnRH administration could be due to the sudden release of LH, leading to ovulation or luteinization of dominant follicles of the present wave (Örsan et al, 2007).

In this respect, overall increase in CLnumber and follicles was 17 CL (28-11 CL) and 21 new follicles (31 from 17 ovulated follicles=14 from 35=21 follicles) post-1st GnRH injection. This means that 1st GnRH resulted in ovulation of 17 mature follicles from total of 31 follicles (remained 14 unovulated follicles) as affected by GnRHLH and initiation of 21 new follicles.

According to luteulyzation of the developed CL post-$PGF_{2}\alpha$ injection (Gordon, 1996) 22 CL were regressed {28 CL+ 4 (35-31 follicles from ovulated follicles)-10 CL}. Finally ovulation of the dominant follicles of the induced (synchronized) follicular wave via the 2nd GnRH injection.

In general, number of CL and follicles may indicate higher ovarian activity of F x R ewes than in F x O ewes.

Table (4): Ovarian structure (right and left ovaries) of treated ewes during treatment period and at and post-AI.

Group		1st GnRH		PGF2α		2nd GnRH		At AI		5 d post-AI	
		CL	F	CL	F	CL	F	CL	F	CL	F
G2	F x R	2	5	4	7	1	6	-	6	6	4
	F x O	1	5	4	5	1	4	-	4	4	3
Total		3	10	8	12	2	10	-	10	10	7
G3	F x R	3	4	5	6	2	5	-	4	4	4
	F x O	1	6	5	4	2	4	-	4	4	3
Total		4	10	10	10	4	9	-	8	8	7
G4	F x R	3	6	7	7	3	7	-	3	3	3
	F x O	1	5	3	6	1	5	-	1	1	3
Total		4	11	10	13	4	12	-	4	4	6
Overall		11	31	28	35	10	31	-	22	22	20

CL: Corpus luteum. F: Follicles

Progesterone profile:

Results presented in Table 5 showed that inspite the significant difference in P4 concentration among groups pre-treatment, ewes in all treatment groups were nearly at similar reproductive status, because P4 concentration was almost less than 1 ng/ml.

On day of 1st GnRH injection (0 time of treatment), P4 concentration elevated in all groups, but the differences among groups were not significant and all P4 concentrations were above 1 ng/ml (Table 5). This was association with the number of CL in treatment groups at this time (Table 4).

Further increases in P4 concentration were recorded in all groups post-1st GnRH injection (on day of PGF2α injection), being significantly ($P<0.05$) lower in G3 than in G2 and G4, but was above 1 ng/ml in in all groups (Table 5).

The protocol of GnRH treatment for ewes has been based on synchronization of follicular growth through ovulation of the dominant follicles and formation of CL post the injection of 1st dose of GnRH and initiation of new follicular growth wave (Gordon, 1996). In accordance with the present results, Beck *et al.* (1996) showed a higher plasma P4 concentration post-treatment with GnRH. Corpora lutea (CLs) secrete P4 later with respect to the LH surge and at a lower rate than CLs formed after subsequent ovulations (Schirar *et al.*, 1989). Such finding is in consistent with the hypothesis that GnRH given on day 5 of the estrous cycle induced ovulation resulting in formation of an accessory CL thereby increasing endogenous P4. This is in agreement with previous report of Howard *et al.* (2006), who found that all cows in the GnRH group developed an additional CL and had greater P4 concentrations by day 13 after AI compared with cows in the saline group.

Complete regression of most CL was suggested in all groups post-PGF2α injection (on day of 2nd GnRH injection) and at AI, whereas P4

concentrations were less than 1 ng/ml in all groups (Table 5) in relation with number of CL on day of 2nd GnRH injection (Table 4). Gordon (1996) reported a luteulyzation of the developed CL by $PGF_{2}\alpha$ injection. Functional luteolysis involves the decline in P4 secretion and begins 3 to 6 hours after the initiation of PGF-2α release (Stabenfeldt and Edquist, 1996).

Finally, P4 concentration showed marked increase 5 days post-AI, indicating incidence of estrus on day of AI and possibility of pregnancy in each group, because P4 concentration increased from <1 ng/ml on day of AI to > 1 n/ml 5 days post-AI. Such results are in relation with absence of CL on day of AI (Table 4). In this respect it was mentioned that ovulation of the dominant follicles of the induced (synchronized) follicular wave via second GnRH injection (Gordon, 1996). High P4 concentrations can lead to high pregnancy and fertility rates (Johnson *et al.*, 1996). It is during the second week of pregnancy that the changes in the uterine secretions are critical for embryo survival (Goff, 2002). Since P4 is essential for maintenance of pregnancy there would appear to be a rationale for its use to improve conception rates and to minimize early embryonic mortality (Inskeep, 2004).

Table (5): Profile of P4 during treatment period in different treatment groups, regardless cross-bred (mean ± SE).

Grp,	Pre-treat. (Day -1)	1st GnRH (Day 0)	PGF2α (Day 5)	2nd GnRH (Day 7	At AI (Day 8)	Post-AI (Day 13)
G2	0.91±0.03[a]	1.07±0.11	1.54±0.15[a]	0.52±0.12	0.15±0.04	1.49±0.11
G3	0.26±0.04[b]	1.01±0.18	1.05±0.12[b]	0.79±0.12	0.26±0.01	1.19±0.13
G4	0.74±0.07[c]	1.14±0.06	1.43±0.08[a]	0.46±0.15	0.11±0.01	1.55±0.12

a, b and c: Means within the same column with different superscripts are significantly different at 5% level.

Reproductive losses:

According to number of CL on day 5 of insemination and number of lamb borns, rate of loss cases in term of unfertilized ova or embryonic mortality was significantly ($P<0.05$) higher in G3 than in G4 (37.5 vs. 25%), while this rate was moderate in G2 (30%) and did not differ significantly from that in G3 and G4. Such finding may be attributed to high dose of inseminates in G4 than in G3 and most of loss cases in G3 may be in term of unfertilized ova.

Generally, overall rate of loss was 31.8% in all treatment groups. When this rate was considered as early fetal mortality, it was

Table (6): Reproductive losses in different treatment groups post-ultrasonic examination on day 5 post-AI.

Treatment group		N	Lambed ewes	Number of lambs(1)	CL No. on day 5 of AI	Losses cases	
						n	%
G2	F x R	5	3	5	6	1	16.7^{b}
	F x O	5	2	2	4	2	50.0^{a}
Total		10	5	7	10	3	30.0^{AB}
G3	F x R	5	1	2	4	2	50.0^{a}
	F x O	5	3	3	4	1	25.0^{b}
Total		10	4	5	8	3	37.5^{A}
G4	F x R	5	1	2	3	1	33.3^{a}
	F x O	5	1	1	1	0	00.0^{b}
Total		10	2	3	4	1	25.0^{B}
Overall		30	11	15	22	7	31.8

a and b: Means within the same column with different superscripts are significantly different at 5% level.

Application of real time- ultrasonography considered practically applicable techniques for simultaneous detection of early pregnancy and embryonic mortality (Shrick and Inskeep, 1993; Kaulfuss *et al.*, 1997). Previous studies using ultrasonography had estimated the incidence of embryonic and or fetal wastages in different species such as sheep (Dixon *et al.*, 2007; Yotov, 2012).

Embryonic and fetal mortality (EFM) are the most important causes of reproductive losses and have a great effect on the fertility of domestic animals (Vanroose *et al.*, 2000). In agreement with the current study, EFM has been estimated to be about 30 % in sheep and goats (Chaudhary and Purohit, 2012). In sheep, it was reported a greater proportion of ewes with partial losses (36.7 %) rather than total losses (24.2 %). Furthermore, the embryonic losses (18.14 %) in the period between day 19-22 to day 40-45 (21-23 days) were higher than the fetal losses in the period between day 40-45 to term (105-150 days) (Dixon *et al.*, 2007). Similar results were reported by many authors (Moraes *et al.*, 2009) on sheep. However, Yotov (2012), showed substantial ($p<0.05$) higher percentages of late embryonic and fetal losses in parous Trakia Merino and Pleven Blackhead sheep (16.7% and 14.1%, respectively) compared to nulliparous animals of the same breed (6.7%; 6.5%) without significant difference in Ile de France breed.

One of the factors that lead to early embryonic mortality in cows is a lower plasma P4 concentration during the post insemination period (Mann and Lamming, 1999). In addition, it has previously been suggested that low P4 during early embryonic development may cause pregnancy failure and thereby reduce the pregnancy rate (Lucy, 2001). Moreover, high P4 after insemination may enhance embryo development and may suppress luteolysis, ultimately resulting in reduced embryonic loss (Mann *et al.*, 1999).

In small ruminant, most embryonic loss has been reported to occur before day 18; when the embryo is still not implanted to the uterine epithelium (referred as early embryonic death) and in turns expensive laboratory

equipment is needed for its diagnosis (Hafez and Hafez, 1993; Bajaj and Sharma, 2011).

In goats, P4 is necessary for pregnancy maintenance, which supplied exclusively from CL (Zamfirescu *et al.*, 2011). The concentration of P4 during placentation might affect late embryonic and fetal survival in cattle (Starbuck *et al.*, 2004), perhaps affecting angiogenesis in the developing embryo or placenta, or both, by altering the levels of vascular endothelial growth factor (Cullinan-Bove and Koos, 1993).

It was reported that serum P4 level decreased below 2 ng/ ml at day 25 post breeding concurrently with the increasing of partial or total EFL (Dixon *et al.*, 2007). Similarly, in cattle and sheep, early embryonic mortality was associated with reduced circulating concentrations of P4 (Mann *et al.*, 1999). This finding may be present in this study for all treatment groups at day 5 post-AI.

On the other hand, in Norwegian dairy goats with fetal losses , it was suggested might be attributed to the abnormal endocrine foetal–placental function not related to function of the corpus luteum and adrenal gland (Engeland *et al.*, 1999). Similarly, Jonker (2004) suggested that P4 measurement is not a useful indicator of fetal compromise or death because P4 concentrations tend to remain elevated until shortly before fetal expulsion, whereas fetal death may have occurred much earlier. In this trend, the sudden drop of pregnancy associated glycoprotein (PAG) values could confirm the occurrence of embryonic mortality in goats (Zamfirescu *et al.*, 2011). Undoubtedly, factors other than reduced circulating concentrations of P4 also contributed to embryonic mortality such as the herd history of fetal loss, abnormal endocrine foetal–placental function (Engeland *et al.*, 1999), and oocyte quality (Campanile *et al.*, 2005).

The impact of economic losses resulting from fetal death include not only the loss of offspring or decreasing the crop yield of calves and kids, but also a prolonged “open” period for the dam leading to increased culling rates (Jonker, 2004).

In conclusion, using GnRH- $PGF_2\alpha$–GnRH protocols (on 0, 5 and 7 d, respectively) and AI 24 h post the 2nd GnRH injection during breeding season give high sufficient of estrous synchronization and lambing , in particular for F x R ewes.

REFERENCES

Abdel-Salam O. M. (2010). Studying the ovarian follicular growth and the role of GnRH in synchronization of ovulation in ewes. M. Sc. Thesis, Fac. Agric., Mansoura Univ.

Ashmawy, T. A. M. (2012). Effect of ovarian synchronization protocols, using GnRH and PGF2α, on ovarian response and reproductive traits of Rahmani ewes. Egyptian J. Sheep & Goat Sci., 7 (2):43- 49.

Ataman M. B. and M. Aköz (2006). GnRH-PGF2α and PGF2α-PGF2α synchronization in Akkaraman cross-breed sheep in the breeding season. Bull. Vet. Inst. Pulawy, 50, 101- 104.

Bajaj, N.K. and N. Sharma (2011). Endocrine causes of early embryonic death. An Overview. Curr. Res. Dairy Sci., 3: 1- 24.

Bane, A. A. (1982). Study on the technique of hemocytometric determination of sperm motility and sperm concentration in bull semen. Cornell. Vet., 42: 518-528.

Beck, N. F. G. ; M. Jones ; B. Davies ; A. R. Peters and S. P. Williams (1996). Oestrus synchronization in ewes: the effect of combining a prostaglandin analogue with a GnRH agonist (buserelin). Animal Sci., 62: 85-87.

Bojanic, S. ; R. Lake ; J. Place ; L. Jones ; J. Laycoock ; G. Carter and J. Alagh-Band-Zadeh (1991). Serum progesterone concentration is raised during early follicular phase in women with polycystic ovaries. Ann. Clin. Biochem., 28: 105-106.

Campanile G. ; G. Neglia ; B. Gasparrini ; G. Galiero ; A. Prandi ; R. Di Palo and M. J. D"Occhio (2005). Embryonic mortality in buffaloes synchronized and mated by AI during the seasonal decline in reproductive function. Theriogenology 63(8):2334-2340.

Chaudhary, A. K. and G. N. Purohit (2012). Ultrasonographic Detection of Early Pregnancy Loss in Dairy Cows. J. Anim. Sci. Adv., 2(8): 706-710.

Christianson, W. T. (1992). Stillbirths, mummies, abortions, and early embryonic death. Vet. Clin. North Am. 8: 623–639.

Culinan-Bove, K. and R. D. Koos (1993). Vascular endothelial growth factor/vascular permeability factor expression in the rate uterus: Rapid stimulation by estrogen correlates with estrogen induced increases in uterine capillary permeability and growth. Endocrionology, 133: 829-837.

Dixon, A. B. (2003). Late embrionic and fetal mortality in the ewes. Ph. D. Thesis: 1-14.

Dixon, A.B. ; M. Knight ; J. L. Winkler ; D. J. Marsh ; J. L. Pate ; M. E. Wilson ; R. A. Dailey ; G. Seidel ; E. K. Inskeep (2007). Patterns of late embryonic and fetal mortality and association with several factors in sheep. J. Anim. Sci., 85: 1274-1287.

Duncan, D. B. (1955). T-test and interval for comparison suggested by the date. Biometrics, 31: 339-359.

Engeland, I.V. ; E. Ropstad ; H. Kindahl ; O. Andresen ; H. Waldeland and A. Tverdal (1999). Foetal loss in dairy goats: function of the adrenal glands, corpus luteum and the foetal–placental unit. Anim. Reprod. Sci., 55, 205–222.

Glazar B. S. ; P. M. McCue ; J. E. Bruemmer and E. L. Squires (2004). Deslorelin on day 8 or 12 postovulation does not luteinize follicles during an artificially maintained diestrus phase in the mare. Theriogenology, 62, 57-64.

Goff, A. K. (2002). Embryonic Signals and Survival. Reprod. Dom. Anim., 37: 133-139.

Gordon, I. (1996). Controlled reproduction in sheep and goat. Dublin, Ireland. pp. 375-397.

Hafez, E. S. E. (1993). Reproductive Cycles. In: Reproduction in Farm Animals. 6th ed. Philadelphia, Lea and Fibeger:94-113.

Halbert, G. W. ; H. Dobson ; J. S. Walton ; P. Sharpe and B. C. Buckrell (1990). Field evaluation of a technique for transcervical intra-uterine insemination of ewes. Theriogenology, 33:1231-1243.

Howard, J. M. ; R. Manzo ; J. C. Dalton ; F. Frago and A. Ahmadzadeh (2006). Conception rates and serum progesterone concentration in dairy cattle administered gonadotropin releasing hormones 5 days after artificial insemination. Anim. Reprod. Sci., 95: 224-233.

Inskeep, E. K. (2004). Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J. Anim. Sci., 82: 24-39.

Jainudeen, M. R. and E. S. E. Hafez (1993). Sheep and goats. In: Reproduction in Farm Animals. E.S.E. Hafez (Ed.) pp. 330-342. Lea and Febiger, Philadelphia, USA.

Johnson, S.K. ; R.A. Dailey ; E.K. Inskeep and P.E. Lewis (1996). Effect of peripheral concentrations of progesterone on follicular growth and Fertility in ewes. Dom. Anim. Endocrinol., 13: 69-79.

Jonker, F. H. (2004). Fetal death: comparative aspects in large domestic animals. Anim. Reprod. Sci., 82-83: 415-430.

Larsson, B. ; A. Gustafsson ; A. Nasholm and L. Bjurström (1991). A programme for oestrus synchronization and embryo transfer in sheep. Reprod. Dom. Anim., 26, 301-308.

Leboeuf, B. ; B. Restall and S. Salamon (2000). Production and storage of goat semen for artificial insemination. Anim. Reprod. Sci., 62: 131-141.

Lucy, M.C. (2001). Reproductive loss in high-producing dairy cattle: where will it end? J. Dairy Sci. 84:1277–1293.

Mann, G. E.; G.E. Lamming; R.S. Robinson and D.C. Wathes (1999). The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. J. Reprod. Fertil., 54: 317–328.

Moraes, E.P.B. ; L. M. Freitas Neto ; C. R. Aguiar Filho ; F. Q. Bezerra ; M. H. Santos ; J. P. Neves ; P. F. Lima and M. A. Oliveira (2009). Mortality determination and gender identification of conceptuses in pregnancies of Santa Ines ovine by ultrasound. S. A. J. Anim. Sci., 39: 307-312.

NRC (2001). National Research Council, Nutrient Requirements of sheep and goats, 7th Rev. Ed. National Academy of Science, Washington, DC.

Örsan, G. ; S. M. Pancarci; M. Çenesiz ; H. B. Beceriklisoy and H. Kanca (2007). Effect of GnRH implant (deslorelin) with different formulation on fertility and delay of oestrous cycle in ewes. Bull. Vet. Inst. Pulawy, 51: 25-29.

Öztürkler Y. ; A. Baykal and B. G. Ven (2003). Combined effect of a prostaglandin analogue and a progestagen treatment for 5 days on oestrus synchronization in Tushin ewes. Indian Vet. J. 80, 917-920.

Peters, A. R. (2005). Veterinary clinical application of GnRH questions of efficacy. Anim. Reprod. Sci., 88: 155-167.

Pursley J.R. ; R. W. Silcox and M. C. Wiltbank (1998). Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. J. Dairy Sci., 81, 2139-2144.

Sallam, A. A. (1999). Physiological studies on reproduction of ewes. Ph. D. Thesis, Fac. Agric., Alex. Univ.

SAS/STAT (2000). Guide for personal computers. Version 8 Ed. Cary NC, USA, SAS Institute.

Schirar, A. ; C. Meusnier; J. Paly; M.C. Lavesseur and J. Martinet (1989). Resumption of ovarian activity in postpartum ewes : role of the uterus. Anim. Reprod. Sci., 19: 79-91.

Stabenfeldt, G. and L. Edquist (1996). Female reproductive process. IN SWENSON, M. & REECE, W. (Eds.) Duke's physiology of domestic animals. 11 ed. London, Cornwell University.

Stevenson, J. S. ; Y. Kobayashi ; M. P. Shipka and Rauchholz, K. C. (1996). Altering conception of dairy cattle by gonadotrophin-releasing hormone preceding luteolysis induced by prostaglandin F2α. J. Dairy Sci., 79: 402-410.

Twagiramungu, H. ; L. A. Guilbault ; J. Proulx and J. J. Dufour (1992). Synchronization of estrus and fertility of beef cattle with two injections of Buserelin and prostaglandin. Theriogenology, 38:1131–114.

Vanroose, G. ; A. de Kruif and A. Van Soom (2000). Embryonic mortality and embryopathogen interactions. Anim. Reprod. Sci., 60-61:131-143.

Yotov S. A. (2012). Ultrasound Diagnostics of Late Embryonic and Foetal Death in Three Sheep Breeds. J. Vet. Adv. 2(3): 120-125.

Zamfirescu, S. ; A. Anghel ; D. Nadolu and N. Dobrin (2011). Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during early pregnancy in Carpathian goat. Annuals of Romanian Society of Cell Biology 16 (2):50-53.

تأثير عدد التلقيحات وتركيز الحيوانات المنوية عند إستخدام التلقيح الصناعى على الاداء التناسلى للنعاج الخليط المعاملة بالجونادوتروفين خلال موسم التلقيح

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تهدف الدراسة الى تقييم تأثير المعاملة الهرمونية (GPG) (صفر ، ٥ ، ٧ يوم) والتلقيح الصناعى خلال ٢٤-٢٨ ساعة بعد الحقنة الثانية من الجونادوتروفين على الاداء التناسلى للنعاج الخليط ، وكذا مقارنة التلقيح مرة واحدة بمرتين بتركيز اقل وتركيز اعلى من الحيوانات المنوية. استخدم ٤٠ نعجة خليط ٢/١ فنلندى رحمانى ، ٢/١ فنلندى اوسيمى خلال موسم تلقيح يناير ٢٠١٢ ، قسمت الحويانات الى اربعة مجاميع (١٠ نعجة بكل مجموعة ، ٥ نعجة من كل سلالة خلط) ، نعاج المجموعة الاولى كنترول (ج١) تم تعريضها للتلقيح طبيعيا ، نعاج المجموعات المعاملة (ج٢ ، ج٣ ، ج٤) حقنت فى اليوم صفر بـ ١ مل رسبتال وبعد ٥ ايام حقنت بـ ٠.٧ مل استروميت ، وفى اليوم ٧ حقنت بـ ١ مل رسبتال وتم التلقيح صناعيا بعد الحقنة الثانية من حقنة الثانية بالرسبتال ، نعاج المجموعات المعاملة اجرى لها نفس المعاملة الهرمونية باختلاف ان تركيز الحيوانات المنوية كان ٣٠٠ × ١٠ ٦ حيوان منوى / مل / مرة بعد ٢٤ ساعة من الحقنة الثانية رسبتال (ج٢) ، وكان ٣٠٠ × ١٠ ٦ حيوان منوى / مل / مرتين بعد ٢٠-٢٨ ساعة من الحقنة الثانية رسبتال (ج٣) ، كان ٤٠٠ × ١٠ ٦ حيوان منوى / مل / مرة بعد ٢٤ ساعة من الحقنة الثانية رسبتال (ج٤). اوضحت النتائج ان معدل الشياع كان اعلى ٧٠% فى ج٢ ، ٦٠% فى ج٣ ، ٤٠% فى ج٤ وبفروق معنوية وكان اعلى فى النعاج خليط الاوسيمى عن خليط الرحمانى. لم توجد فروق معنوية بين المجموعات ولا بين نوعى الخلط المستخدم فى فترة الشبق. معدل الولادة فى النعاج المعاملة تناقص بزيادة عدد مرات التلقيح (مرة مقارنة بمرتين) من ٥٠% (ج٢) الي ٤٠% (ج٣) بينما تناقص من ٤٠ الى ٢٠% بزيادة تركيز الحيوانات المنوية من ٣٠٠ الى ٤٠٠ × ١٠ ٦ حيوان منوى / مل / مرة واحدة (ج٣ ، ج٤ على التوالى). معدل الولادة فى النعاج زاد معنويا بزيادة عدد مرات التلقيح الصناعى ٥٠% مقارنة ب ٦٦.٧% فى ج٢ ، ج٣ على الترتيب بينما تناقص من ٥٠ الى ٤٠% بزيادة تركيز الحيوانات المنوية ج٣ ، ج٤ على الترتيب. كان معدل الولادة فى خليط الرحمانى اعلى من خليط الاوسيمى فى ج٢ ، ج٤ و ج١ على التريب ، كل المجموعات المعاملة اظهرت اقصر فترة ولادة (١-٥ يوم) بينما كانت فى المجموعة المقارنة (ج١) ٣٣ يوم. عدد الحملان فى ج٢ مقارنة بـ ج١ (٧ مولود مقارنة بـ ٦ مولود) ، معدل التوائم كان اعلى فى خليط الرحمانى عن خليط الاوسيمى ، النشاط المبيضى كان اعلى فى ج٢ ، ج٣ عن ج٤ وفى خليط الرحمانى عن خليط الاوسيمى فى اليوم الاول من حقن الرسبتال وزاد تركيز البروجستيرون ثم تناقص بعد حقن الاستروميت وعند التلقيح الصناعى وكان اعلى زيادة بعد ٥ ايام من التلقيح ، الفقد فى الاجنة كان اعلى فى ج٣ عن ج٤ (٣٧.٥ مقارنة بـ ٢٥% على الترتيب) وكان ٣٠% فى ج٢ وكان معدل فقد الاجنة ٣١.٨% فى كل المجموعات المعاملة.

أوضحت الدراسه ان استخدام المعاملة الهرمونية (GPG) (صفر ، ٥ ، ٧ يوم) والتلقيح الصناعى بعد ٢٤ساعة من الحقنة الثانية من الجونادوتروفين خلال موسو التزاوج فى يناير أدى الى أعلى معدل تنظيم للشياع والولادات وأقل فقد تناسلى