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Flushing vs. Hormonal treatment, which better improve the reproductive performance of Ossimi ewes?

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Abstract

The purpose of the current investigation was to evaluate the effect of flushing and hormonal methods on reproductive performance in local Ossimi sheep. Thirty Ossimi ewes of 39.56 ± 0.72 kg average live body weight were used. Animals randomly divided into three groups. The first group (G1) (Flushing without hormonal treatment), the second group (G2) (flushing with injection of GnRH). While, ewes of the third group (G3) flushing and one injection of PGF2α + GnRH. The basal flushing applied by feeding a gradual increased concentrate pelleted mixture, 14% crude protein, started by 500 g/h/day up to 1 kg/h/d during two weeks started prior breeding until the breeding season beside added amount of rice straw. The experiment designed according to the complete randomized design (CRD). There was a significant effect (P≤0.05 or P≤0.01) due to the treatments on litter size at birth (LZB), fecundity rate (FR), net reproductive rate (NRR), birth weight (BW_0), body weight at 10 wks (BW_{10}), total daily gain from birth to 70 d. On the other hand, no significant differences were found due to treatments on laming rate (LR), litter size at weaning (LZW), overall reproductive rate (ORR), livability, body weight at 8 wks (BW_8), and kilograms of lambs born (KLBEL) and weaned (KLWEL). As conclusion, enhancing ration during breeding period is enough to improve the reproductive performance of ewes. The reason of reduced fertility with hormonal treatments may be due to increasing GnRH level against exert inhibitory effects or blocking of GnRH receptors by chemical antagonists of GnRH.

INTRODUCTION

The lambing rate and percent of litter size of ewes are the most important factors for increasing the profit for sheep breeders. Ovarian response and ovulation rate affected by both genetic and environmental factors such as breed, kind of hormones, feeding status, season of the year, and age of ewe and ram. Understanding the fundamentals of folliculogenesis and ovulation rate will ultimately lead to effective protocols for manipulating ovulation rate and improving the overall fertility efficiency of sheep flocks. Also, low heritability of litter size means that change of environmental factors, especially nutrition and hormonal treatments could lead to improve it **(Janssens *et al*., 2004)**.

Nutritional flushing (The regime practice of increasing energy intake) from 3 to 4 weeks before breeding is a routine process applied for many sheep herds to achieve profitability by increasing the multiple birth per ewe. Besides, ewe lambs and ewes that have superior condition are less responsive to flushing than other ewes of the herd. In addition, flushing increases testis size and number of sperms; in consequence it used for rams as well **(Shad *et al*., 2011)**.

Flushing prior the beginning of the breeding season positively affects body condition score (BCS) and improves reproductive performance of goats **(Walkden-Brown and Bocouier, 2000)**. The effect of flushing on growing follicle increase concentrations of glucose, insulin and leptin that acting directly at the ovarian level. The status of follicle development at the time of maximum concentrations of glucose and metabolic hormones may be one of the factors determine whether ovulation rate increases or not in response to nutritional treatment **(Gil, 2003)**.

It is known that increasing nutrition levels influence hypothalamic- pituitary axis, and by that means, effects on gonadotropin releasing hormone (GnRH). Hypothalamic GnRH stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary, which produces ovulation of a large follicle and stimulates luteinization of the follicular remnants. The gonads also secrete other hormones that influence pituitary control of reproduction, such as inhibin, activin, and follistatin. Inhibin and activin are members of the transforming growth factors (TGFs) family **(Scaramuzzi *et al*., 2006)**. GnRH can increase LH and progesterone levels. Progesterone plays an important role during ovulation and embryo implantation at early gestation **(Zare Shahneh *et al*., 2008)** by controlling the estrous cycle of the ewe.

During mating season PGF2α hormone used for controlling the estrous cycle of the ewe through regression of the corpus luteum. Also, using a combination of GnRH-PGF2α found to be effective on the synchronization of estrus in ewes **(Ataman *et al*, 2006 and Ataman and Aköz, 2006)**

In Egypt, some sheep owners don't like multiple birth lambs due to the decrease of birth weight, reduce of growth rates, less survival and increased mortality compared to single lambs, other breeders like twins in their flocks on the basis that ewes have sufficient milk and good care for twin lambs, thus it is possible to improve the previous traits. Considering the fact that Egyptian Ossimi ewes have low twinning rate, the aim of the current research was to evaluate and compare the influence of flushing and hormonal treatment on reproductive performance of local Ossimi sheep.

METERIALS AND METHODS

The study started at the beginning of breeding season (late Dec./Jan.) and lasted to the end of pregnancy. Thirty Ossimi ewes with 39.56 ± 0.72 kg average live body weight were involved in the current study which carried out at Experimental Farm belonging to Animal production Dept., Fac. of Agric., Minia Univ.. Duration of flushing was around two weeks prior breeding and continued two weeks until the breeding season. Two rams were introduced to the ewes for estrus detection and mating for a period of 35 days (two estrus cycles) started at the 3rd week of flushing period. Animals were randomly divided into three groups. The first group (G1), flushing without hormonal treatment, the second group (G2), flushing plus injection of GnRH (1 ml Receptal, Intervet International, B.V. Manufactured in the European Union), the third group (G3), flushing plus injection of both prostaglandin F2α (1 ml Lutalyse$_{TM}$; Pharmacia and Upjohn S.A.) and GnRH (1 ml). The rams received two doses of 10 ml multivitamins (once every 5 days) to increase the concentration of sperms in semen and reduce the percent of abnormal sperms. Animals fed on concentrate feed mixture to cover their nutrient requirements according to live body weight (NRC, 2007). The concentrate feed mixture contained 48 % wheat bran, 17 % yellow corn, 13 % soybean meal (44 % CP), 10.8 % sunflower meal, 4.2 % molasses, 4 % rice hulls, 2 % calcium carbonate and 1 % sodium chloride. Flushing applied by gradual addition of amounts of concentrate pelleted mixture (14% CP) started by 500 g/h/d up to 1 kg/h/d, for one month before breeding season. Rice straw was provided also. Reproductive and productive measurements were calculated as follows:

Lambing rate (%) = (No. of ewes lambed / No of ewes mated) x 100.

Litter size (prolificacy) = (No. of lambs born / No of ewes lambed) x 100

Fecundity rate = lambs born/ ewes mated.

Livability: (No. of lambs that remained alive / No. of lambs born**).**

Weaned rate (%) = (No. of lambs weaned / No of lambs born) x 100.

Overall Reproductive Rate (ORR) = No. of lambs weaned/No. of ewes mated.

Net Reproductive Rate (NRR) = Total weight of lambs weaned/ ewes mated.

KLBEL: (Kilograms of lambs born/ No. of lambs born) and

KLWEL: (Kilograms of lambs weaned/ No. of lambs weaned).

Statistical analysis of data

The experiment was designed according to the complete randomized design (CRD). Data were analyzed statistically by ANOVA methods, SAS (2006). Significant differences ($P \leq 0.05$) between means tested using Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

The effect of flushing and flushing plus hormonal treatment on reproductive traits of ewes are shown in Table (1).

Significant effects (P≤ 0.05 or P≤ 0.01) for treatments on litter size at birth, fecundity rate and net reproduction rate (NRR?) were observed. While, no statistically significant differences were noticed for mating and lambing weights of ewes, lambing rate, litter size at weaning and overall reproductive rates among studied groups.

Table (1): Means ± Std (or SE?) for effect of treatments on reproductive traits of ewes.

Item	Control (Flushing)	GnRH + (Flushing)	GnRH+ PGF2α+ (Flushing)	Overall mean	Level of Sig.
MW	41.33±4.09	39.64±3.21	37.67±3.01	39.56±3.79	NS
LW	54.25±5.85	52.00±4.86	48.61±4.36	51.68±5.56	NS
LR	0.88±0.15 (8/9)	0.78±0.16 (7/9)	0.75±0.13 (9/12)	0.80±0.08 (24/30)	NS
FR	1.22±0.32a (11/9)	0.88±0.21b (8/9)	0.75±0.17b (9/12)	0.95±0.35 (28/30)	**
LZB	1.37±0.44a (11/8)	1.14±0.38 ab (8/7)	1.00±0.0b (9/9)	1.17±0.33 (28/24)	**
LZW	1.12±0.21 (9/8)	1.00±0.11 (7/7)	1.00±0.0 (9/9)	1.04±0.18 (25/24)	NS
ORR	0.88±0.15	0.78±0.17	0.77±0.11	0.81±0.08	NS
NRR	13.64±5.06a	11.36±3.50b	14.89±2.68a	13.30±5.95	*

MW: Mating weight; **LW**: Lambing weight; **LR**: Lambing rate; **LZB**: litter size at birth; **LZW**: Litter size at weaning; **FR**: Fecundity rate **ORR**: Overall reproduction rate; **NRR**: Net Reprod. Rate NS: Not significant;
* Significant at 5% (P≤ 0.05); ****** Highly significant at 1% (P≤ 0.05) and **a, b, & c** means with the same letter are not significantly difference.

General mean of lambing rate (LR) was 0.80, although the effect of treatments on this trait was not significant. However, the best value was 0.88 for (G1) followed by 0.78 for (G2) and the least one was 0.75 for (G3). A low LR means that some ewes did not lamb, for any cause. This is accordingly an indicator for the fertility perspective of a herd. **Zonturlu *et al.* (2018)** found no significant differences in terms of lambing rate of Awassi ewes among three groups; control, one injection of GnRH and two doses of GnRH (at the time of mating and 9 days later).

The ewes in control group had more twins (P<0.01) as they had a higher total number of lambs born (1.37) than those hormonally treated (1.14 for G2 and 1.00 for G3).

In Egypt, **Abdalla *et al.*, (2014)** found that the litter size of Barki ewes were 1.05, 1.38 and 1.00 for control, ewes received PGF2α + PMSG and ewes received PGF2α + GnRH treatments, respectively.

The same trend was observed in the present study for fecundity rate (P<0.01), the highest value was 1.22 for G1 compared to 0.88 and 0.75 for G2 and G3, respectively. **Rekik *et al*. (2016)** noticed that litter size and litter weight at lambing were unaffected by treatment group (p > .05). No multiple births occurred in the sheep treated with injections of GnRH analog on days 0 and 10 or a single injection of PGF2α analog dinoprost on day 6.

On the other hand, treatments had no significant effect on both LZW and ORR. The highest values of LZW and ORR were 1.12 and 0.88 for G1 compared to 1.0 & 0.78 and 1.0 & 0.77 for G2 and G3, respectively. The ORR is a very complex trait, having many components as conception rate x fecundity x lambs' survival.

A significant effect of treatment (P<0.05) was observed for NRR, the highest value was 14.89 for G3 compared to 13.64 and 11.36 for G1 and G2, respectively. This trait is a product of overall reproduction rate x average weaning weight and thus reflects growth rate as well as reproductive rate.

Marzouk (1997) noticed that the values of overall reproduction rate and net reproductive rate for Ossimi ewes (with flushing) were 0.58 and 8.15 kg, respectively.

In general, the increased level of nutrition can affect gonadotropins secretion, progesterone, oestradiol, insulin and growth hormone, ovulation rate and litter size. Also, specific components of the diet, like vit. E and selenium, may have a positive effect on increasing semen quality and quantity. The effect of diet of the rams on reproductive success of ewes after service needs to be evaluated **(Yue *et al*., 2010)**.

Somchit (2011) noticed that evaluation of influence of feeding on ovulation rate have been assigned by three types of effects; immovable, mobile and straight up . Nutrition affected fertility efficiency at germinated levels through the circulating metabolic hormones which control follicle growth and play an intermediate role on ovulation rate, through regulating the reproductive hormones such as gonadotrophins in follicles.

Assavacheep (2011) reported that energetic materials act as metabolic signals to the reproductive system, involved within the hypothalamic pituitary axis and increase the GnRH hormone. Increasing energy intake raise blood glucose (a major source of energy for the ovary) and insulin which increase the pulsatile LH secretion and improve ovarian response to LH stimulation. While the positive energy balance raises the plasma level of insulin growth factor-1 which is critical to ovarian follicular development and ovulation rate.

On the other hand, the use of GnRH in combination with other hormones has been reported in many researches which improved the prolificacy and fertility efficiency of ewes **(Beck *et al*., 1996; Karaca *et al*., 2009 and Mirzaei *et al*., 2014)**. The physiological basis for using GnRH and its analogs is that the injection of this hormone can result in ovulation by stimulating LH release **(Twagiramungu *et al*.,1995)**. **Beck *et al.* (1996)** noticed the induction of high fertility during breeding season on ewes received injection of GnRH 5 days prior prostaglandin F2α (PGF2a) analogue treatment.

Besides, the difference in the response was due to variations in ovarian condition among the ewes at time of the treatment **(Rubianes *et al*., 2003)**. The day of the cycle, at which PGF2α was given, affects the time interval for the start of oestrus **(Houghton *et al*., 1995)**. The response to GnRH depends on the moment of the cycle at which the hormone is administered **(Geary *et al*., 2000)**.

The GnRH administration on the day of mating improved the reproductive performance in Lohi sheep. GnRH may be luteotrophic or embryotrophic and thereby could improve the embryo survival **(Mushtaq and Zahida , 2010)**.

The findings of the current study that control group (flushing) was better than hormonal treated groups can be explained on the basis of the three factors might interfere with fertility through manipulating the GnRH stimulation of the pituitary gland:(1) GnRH can be prevented from reaching its pituitary receptors by neutralization of GnRH in the hypophysial portal blood by antibodies; (2) the GnRH receptors can be blocked by chemical antagonists of GnRH; and (3) GnRH agonists exert inhibitory effects when given chronically **(Fraser, 1982)**. Moreover, **Amanda *et al*. (2012)** reported that administration of GnRH 24 hours after sponge withdrawal did not improve ovulation or pregnancy of ewes subjected to a short-term synchronization of estrus.

The case of follicle development at the time of highest concentration of glucose and metabolic hormones may be one of the factors that evaluate whether ovulation rate raised or not in response to nutritional treatment.

It is difficult to make comparison either among trials in different countries or different herds in the same country concerning estrus response, ovulation and fertility traits. This is due to variations in breed (genetic) and environmental factors. For example: a hormone protocol (type and doses) such as combination of GnRH with various hormones used in a range of methods, doses and times of injection may be

Table (2): Means ± SE for effect of treatments on livability and growth performance of lambs.

Item	Control (Flushing)	GnRH + (Flushing)	GnRH+ PF2α+ (Flushing)	Overall mean	Level of Sig.
Livability	0.82±0.11	0.88±0.14	1.00±0.0	0.89±0.06	NS
BW_0	3.46±0.53b	3.60±0.44ab	4.14±0.59a	3.75±0.61	*
BW_8	13.64 ± 2.75	14.61 ±2.82	14.99 ± 2.26	14.47 ±2..80	NS
BW_{10}	15.33±2.19b	16.97±2.80a	18.16±2.75a	16.85±3.34	**
ADG	0.167±0.02b	0.178±0.03ab	0.190±0.05a	0.179±0.05	*
KgB	5.34±1.32	4.16±1.81	4.14±0.59	4.56±1.26	NS
KgW	15.65±2.96	14.61±2.82	14.99±2.26	15.12±3.10	NS

BW: Birth weight BW_8 : Eight weeks body weight BW_{10} :Ten weeks body weight ADG: Average daily Gain from BW_0 to 10 wks weight. KgB: Kilograms of lambs born and KgW: Kilograms of lambs weaned.
NS: not significant; ***** : Significant at 5% ($P \leq 0.05$); ** : Highly significant at 1% ($P \leq 0.01$) and means with the same letter (**a, b, or c)** are not significantly differ.

the reason of these discordant in the findings of the previous researches.

The effect of flushing and flushing plus hormonal treatments on livability and growth performance of lambs are illustrated in Table (2).

Treatments had a significant effect ($P \leq 0.05$ or $P \leq 0.01$) on BW_0, BW_{10}, TDG and KLWEL. While, no significant differences were observed in livability, BW_8 and KLBEL among different groups.

Lambs crop is an important profitable indicator for sheep owners. It depends on both prolificacy and birth weight. The value of overall livability percent. of lambs in current study was 0.89. Although the effect of treatments on livability (from birth to 8 weeks) was not significant, it can be observed that G3 ewes had the highest value (1.00) for this trait followed by ewes in G2 (0.88) and ewes in G1 (0.82). A probable reason for this result is that all lambs in G3 were born single, consequently had a heavier birth weight compare to other groups. On the other hand, the lambs in G1 had greater twins and those lambs had a lighter average birth weight and slower growth rate than lambs born single.

The livability differ according to type of birth, birth weight, adequacy of colostrum received by lambs' rearing system, season of the year and veterinary care. Survival rates of twins were markedly lower than singles. The result of current research support by **Oldham *et al*. (2011)** who reported that birth type of lambs and light weight at birth were the greatest risk factors regarding lamb mortality. To increase lamb livability or survival, it is important to manage twin bearers separately and give them enough starter or replacements in case of early weaning and to maintain enough milk at late weaning (3 mo.).

Average BW of lambs born from G1 ewes (3.46 kg) was lower than those from G2 ewes (3.60 kg) and G3 ewes (4.14 kg). Thus, more lambs produced per ewe associate with production of lambs with lower weights. **Hassan and Marzouk (1997)** reported that weight of multiple birth lambs was significantly lighter than the single birth one (P <0.01) at all ages studied. **Alifakiotis (1986)** reported that the cause of discordant results reported by different researchers on lamb birth weight can be explained by the differences in type of birth, sex, age of dam, management system, nutrition of pregnant ewes, body condition, and breed of animals.

A highly significant effect ($P \leq 0.01$) was observed on body weights at 10 wks, the highest values were 18.16 kg for lambs in G3 followed by 16.97 kg for lambs in G2, while the control group had the lowest value, 15.33kg. The same trend was noticed for effect of treatments on

TDG. The highest value was 0.190 kg for lambs in G3 and the lowest was 0.167 kg for lambs in G1 and the lambs in G2 came in between (0.178 kg).

On the other hand, no significant differences were observed for treatments on both KLBEL or KLWEL traits. Although, this effect was not significant, but it can observe that the highest values were 5.34 and 15.65 kg for lambs in G1 and the lowest were 4.14 and 14.99 kg for lambs in G3 while lambs in G2 came in between (4.16 and 14.61 kg), respectively.

The reason for increasing kilograms weaned of lambs in the control group (G1) can be attributed to the high rate of multiple births consequently a greater number of lambs born weaned.

The findings in present study are in agreement with observations reported by **Marzouk (1997)** who found that the values of overall livability, daily gain (from birth to 8 wks), KgB and KgW of Ossimi lambs were 0.92, 0.204 kg, 5.50 kg and 16.98 kg, respectively. Similar result was obtained by **Morsy (2002)** on the same breed and the same condition.

It could concludethat enhancing level of feeding prior breeding period was enough to improve the reproductive performance of ewes. The less fertility of hormonal treated groups compared to control group in this study may due to that increaseing GnRH level exert agonist inhibitory effects or blocked GnRH receptors by chemical antagonists for GnRH or that the condition of ovaries at time of receiving the hormone leads to differences in the response of ewes when start the oestrus cycle. Besides, it is better to repeat the experiment using a larger number of ewes and in different seasons to confirm these result**s.**

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