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Increasing ovulation quota: more than a matter of energy

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ABSTRACT

***Background*:** Nutritional supplementation before breeding (Flushing) has become a common practice and is a reliable method to improve lambing and twining rates in sheep. The improvement of the body condition of a ewe is reflected in a higher number of ovulatory follicles and is termed "static effect of nutrition". Shorter periods of nutritional supplementation can also affect follicular development in the absence of changes in the body condition and weight of the animal, which is known as "acute effect of nutrition". Studies of follicular development in small ruminants have shown that 4 to 5 follicular waves occur during the estrous cycle, and that waves emerge every 5 to 7 days. However, the selection phase of the follicular wave occurs within 3 to 4 days, thus the length of flushing could last merely the time needed to push follicles over the selection threshold.

***Review*:** Here we examined the evidence produced by our research on the minimum length and appropriate timing of nutrient supplementation needed to enhance ovulation rate and prolificacy in sheep. Ewes have follicles ready to reach ovulatory size at any time of the estrous cycle and, when a follicular phase is induced, most show estrus and ovulate within 60 to 80 h. Hence, ovulatory follicles should commit for ovulation shortly after the decline in progesterone if they are to achieve ovulatory competence. We showed that an ultrashort flushing (USF) given as a single administration of a glycogenic substance at the time of prostaglandin-induced luteolysis (Control =1.6 ± 0.06 vs. USF= 2.08± 0.06) or progestin withdrawal (Control =1.64 ± 0.07 vs. USF= 2.41± 0.09) increased ovulation rate ($P < 0.01$). This increase was associated with elevated glucose and insulin concentrations for 12 h after USF ($P < 0.01$). However, the diameter of the three largest follicles did not change between the day of flushing and the day of estrus and did not differ between the control and the USF ($P > 0.10$). The USF could act either by advancing follicle maturation, or by affecting the feedback loop between the ovaries and gonadotrophin secretion. Therefore, we measured mRNA abundance for LH receptor (LHr), 3β-hydroxysteroid dehydrogenase (3β-HSD) and P450 aromatase at 0, 12, 24 and 48 h after the start of luteolysis in ewes treated or not with USF. Aromatase mRNA decreased in large follicles 12 h after USF ($P < 0.01$), with no changes on mRNA for LHr or 3β-HSD. Further, we observed that the *in vitro* rumen fermentation and the *in vivo* glucose plasma concentrations in response to an isoenergetic (1470.83 kcal) single oral drench of glycerol, propylene glycol or molasses differed. The longer time to begin fermentation of glycerol allows for its absorption and its direct use for glucose production in the liver. Molasses was preferably fermented to butyrate, whereas propylene glycol was preferably fermented to propionic acid thus serving as glycogenic substrate. Glycerol and propylene glycol increased glucose and insulin concentrations *in vivo*, whereas molasses did not, thus the later may not be suitable for the USF. An increase in ovulation rate and prolificacy was also obtained with treatments that cause nutrient redistribution in the animal such as beta adrenergic receptor agonists and bovine somatotropin.

***Conclusions*:** We have developed an ultrashort flushing with glycogenic solutions that when applied at luteolysis will increase ovulation rate in sheep. This hyper-acute effect of nutrition caused a reduction in mRNA for P450 aromatase 12 hours after the glycogenic drench. In addition, from our results we can infer that ovine follicles can develop ovulatory capacity within 48 h after being selected when coinciding with the follicular phase.

Keywords: flushing, ovulation rate, nutrition, sheep.

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I. INTRODUCTION

Flushing is one of the oldest reproductive technologies known to improve reproductive performance and to enhance the lambing rate of sheep and goat flocks [20,34]. The first scientific report of flushing in 1899 [11], describes that sheep that are heavier or with a higher nutritional intake have a higher proportion of twin lambings. Subsequent studies have confirmed that as body weight of the ewe increases, there is also an increase in the twinning rate [6] and in the ovulatory quota [24]. This effect of nutrition on ovulation rate that is associated with elevated body weight is termed "static effect of nutrition" [33,39]. Traditionally, the recommendation for animal husbandry is to improve the feeding in quantity and/or quality of the sheep somewhat around 2 months to 3 weeks prior to the breeding season, in an attempt to improve the body condition of the sheep and thus achieve higher lambing rates and prolificacy. In the tropics, where high quality pastures are not available, sheep need to be fed nutritional supplements containing proteic or energy loaded components to improve these parameters. However, this approach is rather time consuming and in some instances the cost could make it impractical.

Food supplementation may acutely increase ovulation rate without affecting the body condition or the weight of the animal. This effect is termed "acute effect of nutrition" where supplementation given for only 4 to 9 days can increase ovulation rate [19,36]. Studies of follicular development in small ruminants have shown that 4 to 5 follicular waves occur during the estrous cycle, and that waves emerge every 5 to 7 days [1,18]. The selection phase of the follicular wave lasts only 3 to 4 days, thus, if ovulatory follicles are to be chosen from the pool of growing follicles, flushing may not need to last more than the length of a follicular wave.

To get an insight into the mechanism governing the selection of ovulatory follicles and ovulation rate as affected by nutritional influences, we have used a model where flushing is given via glycogenic solutions. Glycerol and propylene glycol are substances often used as energy additives in ruminant nutrition for their glycogenic and anti-ketogenic properties [17,31]. In dairy cows, their addition in early lactation diets decreased free fatty acidβ and β-hydroxybutyrate whilst increasing blood glucose concentrations [5,27].

In this manuscript we will examine the evidence produced by our research regarding the minimum length of the nutritional supplementation needed to enhance ovulation rate and prolificacy, and the appropriate timing for nutrient supplementation.

II. LENGTH AND TIMING OF THE FLUSHING TREATMENT

Recent studies have shortened the length of flushing establishing a span of supplementation between nine and four days prior to breeding. However, the minimum length of flushing needed to increase ovulation rate is still to be determined. The answer to this question could depend on the time that a follicle needs to grow and mature to reach an ovulatory status. In small ruminants, the follicular wave is no more than 6 to 7 days long, and the ovulatory follicle is selected only a few days before ovulation [32,35] Thus, it appears that in these species, an ovulatory follicle could grow and develop to reach ovulatory capacity within a short period of time. We therefore tested whether a short flushing, starting three days prior to the end of the luteal phase, would suffice to induce an increase in ovulation rate. Following this protocol, we administered glycerol (100 mL, *tid*) as a glucogenic substrate and found that glycerol drench increased both glucose and insulin concentrations, and the number of ovulating follicles form 1.2 to 1.6 [30]. Similar results have been obtained by other investigators after short flushing treatments with oral glycogenic substances [19], intravenous infusions of glucose [13,25] or glucosamine [25], or by adding lupin to the diet [8,26]. It appears that the increase in ovulation rate is related to an increase in glucose, insulin and leptin concentrations without detectable changes in IGF-I or FSH [38].

Although the follicular waves in sheep and goats, as for other ruminants, have a recruitment phase where a pool of follicles respond to FSH, and further a selection and dominant phases, their waves are not as clearly spaced as in cattle and seem to overlap in time. In addition, sheep and goats appear to have follicles prepared to reach ovulatory size at any time of the estrous cycle, since when a follicular phase is induced by prostaglandin

administration or by the withdrawal of a progestin treatment, most sheep show estrus and ovulate within 60 to 80 h respectively. Furthermore, the ovulatory follicles could originate from the last, second last or both waves of follicular development [18]. In addition, when follicles that ovulated derived from the last and second last follicular waves, the second follicle was selected within the previous two days [18]. Therefore, this evidence allows us to speculate that in small ruminants: 1) The dominance effected by the largest follicle does not completely suppress the development of new follicles up to a gonadotrophin dependent stage; 2) Regardless of the stage of the oestrous cycle, sheep have follicles well advanced in the developmental process, capable to grow and mature within a very short period of time and ovulate if the adequate endocrine stimuli is encountered and 3) A second ovulatory follicle could be drawn from the latest follicular wave even in the presence of a dominant follicle.

These observations led us to believe that the period needed to achieve an increase in ovulation rate by flushing could be further shortened if given at a time where follicles predestined for ovulation are selected. The precise moment for this stimulus seems to be the time where the LH pulse frequency increases after the decline in progesterone concentrations (*i.e*. the start of luteolysis). However, the strength and duration of the stimulus remains to be determined. Ovulatory follicles should commit for ovulation shortly after the decline in progesterone if they are to achieve ovulatory competence. Consequently, we hypothesized that the window of time where the follicle is selected for ovulation occurs within the first 12 hours after the initiation of luteolysis.

A short nutritional stimulus that effectively enhances ovulation rate would necessarily be acting either directly at the ovarian level or through hormones and factors other than gonadotropins, whose concentrations would be directly affected by the particular given nutrient. We chose to use oral glycerol as the flushing stimulus since it can be administered as a drench, it provides a reliable source of quickly available energy, and is short lived, ensuring that the stimulus is focused in a limited and defined period of time. We characterized the peripheral concentrations of glycerol, glucose and insulin and the ovulation rate in response to this ultrashort flushing (USF) in sheep whose estrous cycles were synchronized either with prostaglandin F2α or progestin protocols. The ovaries of these sheep were scanned by ultrasonography, and animals bearing a corpus luteum were treated with PGF2α and assigned randomly either to a control group or to a group where the USF was given as a single drench of 300 mL glycerol (90% glycerol: 10% water) at the time of PGF2α injection. Estrus was detected by a vasectomized ram and ovulation was estimated by counting the number of corpora lutea by ultrasound scanning seven days later. Ultrashort flushing, at the time of luteolysis, increased the ovulation rate of the sheep with 89% of ewes having multiple ovulations, and 17.5% with three or more ovulations ($P < 0.01$; Table 1) [22].

Progestin containing devices are a common method for the synchronization of estrus in sheep. However, treatment with progestins to synchronize the estrous cycle, when in the absence of the CL as a natural source of progesterone, modifies follicular development in cattle [3], and sheep [10], inducing the formation of persistent dominant follicles. Therefore, we tested the effect of the USF in sheep synchronized with fluorogestone acetate containing intravaginal sponges [22]. Ultrashort flushing at the time of pessary withdrawal increased the proportion of ewes with multiple ovulations ($P < 0.01$), 43% ovulating three or more follicles, 51% with double ovulations and only 6% with single ovulations (Table 2). In addition, blood concentrations of glycerol and glucose increased ($P < 0.01$) immediately after USF and remained elevated for at least 10h post-treatment (Figure 1). However, there were no differences in

Table 1. Ovulation type and ovulation rate in Pelibuey ewes following ultrashort flushing (USF) with a single oral administration of 300 ml of a glycogenic solution (glycerol: water; 90:10 v/v) at estrous synchronization with PGF2α.

Treatment Group	n	Ovulation type (%)				Ovulation rate
		Single	Double	Triple	Quadruple	
Control[a]	58	39.66	60.34	0	0	1.6 ± 0.06 [a]
USF[b]	74	10.81	71.62	16.22	1.35	2.08 ± 0.06 [b]

[a,b]Different superscripts within a column differ ($P < 0.01$).

glucose, insulin and IGF-I concentrations 24 h after flushing compared to their respective concentrations before drenching [22].

In a further study, the follicular dynamics were assessed in 12 sheep by ultrasound scanning. On day 10 of the estrous cycle ewes were injected with PGF2α and treated with USF as previously described. Ultrasound scanning of the ovaries was carried out from day one of the previous cycle and continued until the next estrus occurred. The number of small (<4mm) and large (>4mm) follicles was not affected by USF. Similarly, the diameter of the three largest follicles present in the ovaries at the time of estrus was not different between treatments and there were no changes in the diameters of these follicles between the day of flushing and the day of estrus [22].

This hyper-acute effect of flushing demonstrates that the time of luteolysis is a period where follicles could be selected for ovulation concomitant with a transient increase in glucose lasting for at least 10 h, but less than 24 h, that suffice to stimulate the selection of more than

Table 2. Ovulation type and ovulation rate in Pelibuey ewes following ultrashort flushing (USF) with a single oral administration of 300 ml of a glycogenic solution (glycerol: water; 90:10 v/v) after estrous synchronization with FGA+ PGF2α.

Treatment Group	n	Ovulation type (%)				Ovulation rate
		Single	Double	Triple	Quadruple	
Control[a]	55	39.62	56.60	3.77	0	1.64 ± 0.07[a]
USF[b]	53	5.66	50.94	39.62	3.77	2.41 ± 0.09[b]

[a,b]Different superscripts within a column differ ($P < 0.01$).

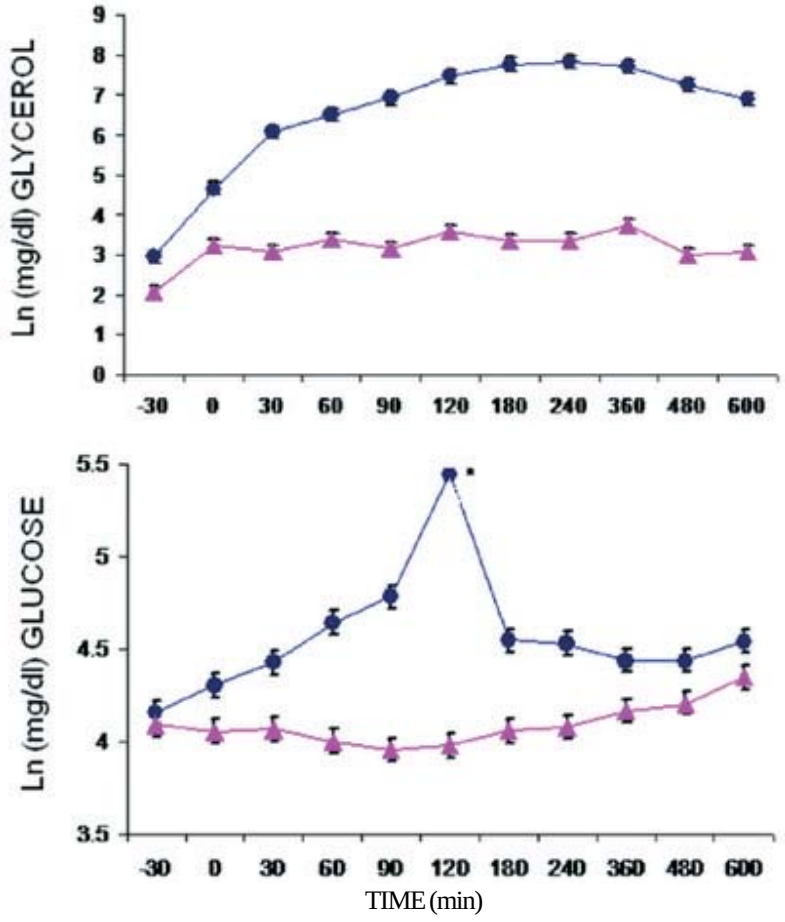

Figure 1. Blood concentrations of glycerol and glucose in control and USF (300 mL of a 90% glycerol drench) treated ewes, from 30 min before to 600 min after treatment. *Values over the detection limit of the assay.

one follicle and increase the ovulation rate.

III. MECHANISMS BY WHICH THE ULTRA SHORT FLUSHING ENHANCES THE OVULATION RATE

Scarramuzzi *et al.* [33] revised the possible mechanisms of action of the acute effect of flushing on ovulation rate and proposed a feedback loop between the ovaries and gonadotrophin secretion, where nutrition causes a direct inhibition of follicular estradiol production leading to compensatory secretion of FSH that stimulates folliculogenesis. Alternatively, insulin could advance the maturation of the follicle that would be reflected in increased expression of LH receptor (LHr) and 3β-hydroxysteroid dehydrogenase (3β-HSD) mRNA expression [9].

To test these hypotheses the estrus cycle of 30 ewes was synchronized with progestin intravaginal sponges and prostaglandins. Animals were given the USF on the day of progestin withdrawal. The ovaries were removed surgically before treatment (time 0; n=6), or at 12, 24 and 48 h after being treated with either glycerol or water (n=4 per treatment by time category). The ovarian follicles were dissected out and counted. Follicles larger than 3mm in diameter were pooled and the mRNA extracted for specific P450aromatase, 3β-hydroxysteroid dehydrogenase (3β-HSD) and LH receptor (LHr) determination. Ultrashort flushing increased the number of follicles larger than 3mm at 48 h after treatment. No effect was observed in small follicles. In large follicles, aromatase mRNA abundance decreased ($P < 0.01$) 12 h after treatment (Figure 2). There was no effect of treatment on mRNA for LH receptor or 3β-HSD (Figure 2) [9].

These results demonstrate a reduction in aromatase expression in potential ovulatory follicles twelve hours after flushing. The decrease in aromatase may favour a transient increase in FSH concentrations that would allow the stimulation and selection of supplementary ovulatory follicles. In addition, it shows that the twelve hours that follow luteolysis are fundamental in the selection of ovulatory follicles and in the determination of the ovulatory quota in sheep.

IV. ARE ALL GLYCOGENIC SUBSTANCES EQUAL FOR THE ULTRASHORT FLUSHING?

Both glycerol and propylene glycol when given to ruminants serve as energy substrates [27, 29]. *In vitro*, glycerol and propylene glycol are fermented and transformed to propionic acid [17,31]. Propionic acid is converted to glucose *in vivo* [2,12]. Propylene glycol can be obtained from glycerol [37] and it is frequently used as feeding additive in dairy cows during early lactation in order to increase blood concentrations of propionic acid, glucose and insulin [5,27]. Molasses, is a byproduct of the sugar cane industry, which is widely used in ruminants as a source of soluble carbohydrates rapidly fermentable in the rumen [40]. The effects of molasses on VFA production is related to its level in the diet. Low dietary inclusion of molasses (<15% DM) does not modify the fermentation pattern of the rumen and VFA production is similar to that observed with grain diets [21] with a desirable production of propionate as gluconeogenic substrate for the liver. In contrast, when molasses inclusion exceeds fifteen percent, propionate production is reduced and butyric acid is increased [23,28].

Fermentation of feedstuffs by rumen microbes produces gas at a rate that is directly proportional to its degradation in the rumen. Both the rate of gas production

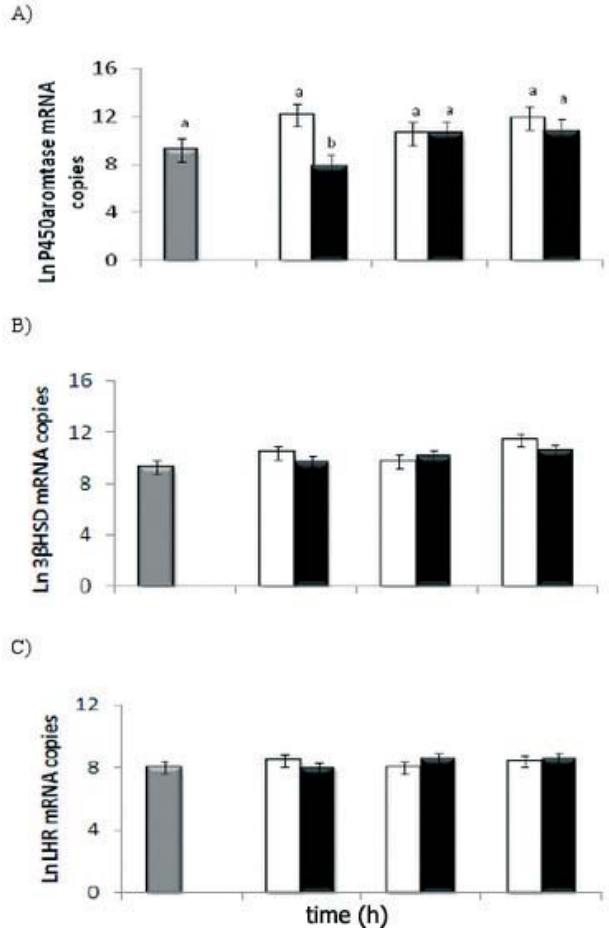

Figure 2. Effect of ultrashort flushing (black bars) or control (empty bars) on number of copies of mRNA expressed for (A) P450aromatase, (B) LHr and (C) 3βHSD. [ab]Indicate differences within a time point ($P < 0.01$).

and the fatty acid preferably produced after fermentation may alter the glycogenic capacity of the substrate. We studied the *in vitro* rumen fermentation and the *in vivo* response of sheep to an isoenergetic (1470.83 kcal) single oral drench of glycerol, propylene glycol or molasses. We observed that glycerol and propylene glycol increased ($P < 0.01$) the proportion of propionate production *in vitro* [9]. Glycerol remained unfermented for the longest time as shown by the greater lag time (10h; $P < 0.05$) and molasses showed a fastest rate of gas production and ($P < 0.05$) the shortest time to be fermented (Table 3). *In vivo*, glycerol and propylene glycol increased glucose plasma concentration for up to 12 h after treatment (Figure 3). Insulin secretion increased for a period of 12 h in the glycerol and propylene glycol groups ($P < 0.01$). In contrast, molasses caused a mild increase in both glucose and insulin that lasted for less than 60 and 12 min respectively (Figure 3) [9].

Hence, the results of this trial showed important differences in the fermentation and glycogenic responses to these three energetic substrates. The longer lag time of glycerol allows for its absorption and its direct use for glucose production by the liver. Propylene glycol has a similar lag time than molasses, but it is preferably fermented to propionic acid thus serving as glycogenic substrate. Thus, glycerol and propylene glycol cause a large and sustained increase in insulin concentrations that together with glucose may enhance ovulation rate.

V. WHAT IS THE MINIMUM DOSE NEEDED TO INCREASE OVULATION RATE WITH AN ULTRASHORT FLUSING?

So far, we have established that USF with a single drench of 300 ml of a 90% glycerol solution coinciding with the initiation of luteolysis augments the number of ovulations and is associated with a 12h increase in glucose and insulin. In addition, this increase in ovulation rate is related to reduced aromatase mRNA abundance in the ovulatory follicles at 12 h after treatment. Thus, we have limited the time frame where follicles are selected to add to the ovulation quota within a 12h period after luteolysis. However, this period of time could actually be shorter than the period indicated by the results already shown. In all cases, the ultrashort flushing does not alter the body condition score or weight of the ewe and seems to act by its effects on insulin and glucose concentrations rather than the net amount of energy given to the animal.

Although flushing can be limited to a single glycogenic drench at the time of luteolysis, we observed as much as 43% of superovulated animals with the 300 mL glycerol dose (Table 2). Thus, the determination of a minimum flushing dose could help reduce the incidence of triple and quadruple ovulations. One hundred and twenty sheep were scanned by ultrasonography to determine their ovulation rate, and were randomly divided into four groups of 30 ewes each that received 300, 200, 100 or 50 mL of glycerol at the time of PGF2α injection. Flushing at all the doses tested increased ovulation rate ($P < 0.01$). A hundred milliliters of glycerol solution caused the highest ovulation rate. However, doses of 100 mL or above superovulated between 17 and 25% of the sheep (Table 4). Thus, it appears that ultrashort flushing with 50 mL dose is sufficient to cause an increase in ovulation rate without the hindrance of superovulation in sheep [22].

VI. OTHER MANIPULATIONS TO INCREASE OVULATION RATE

The static effect of flushing on ovulation quota is apparently due to an increase in the number of

Table 3. Cumulative gas production and estimated kinetic parameter model for liquid energetic sources.

Substrate	Maximum volume (Y), mL	Rate of gas production (s), h^{-1}	Lag time (L), h
Glycerol 320 µL	178.4[b]	0.024[e]	10.23[b]
Propylene glycol 320µL	22.6[d]	0.062[c]	1.81[d]
Molasses 320 µL	161.5[cb]	0.085[a]	1.86[d]
SEM	4.03	0.001	0.12
R^2	0.98	0.98	0.99
P value of regression model	0.0001	0.0001	0.0001

[abc]Means within column with different superscript differ ($P < 0.05$).

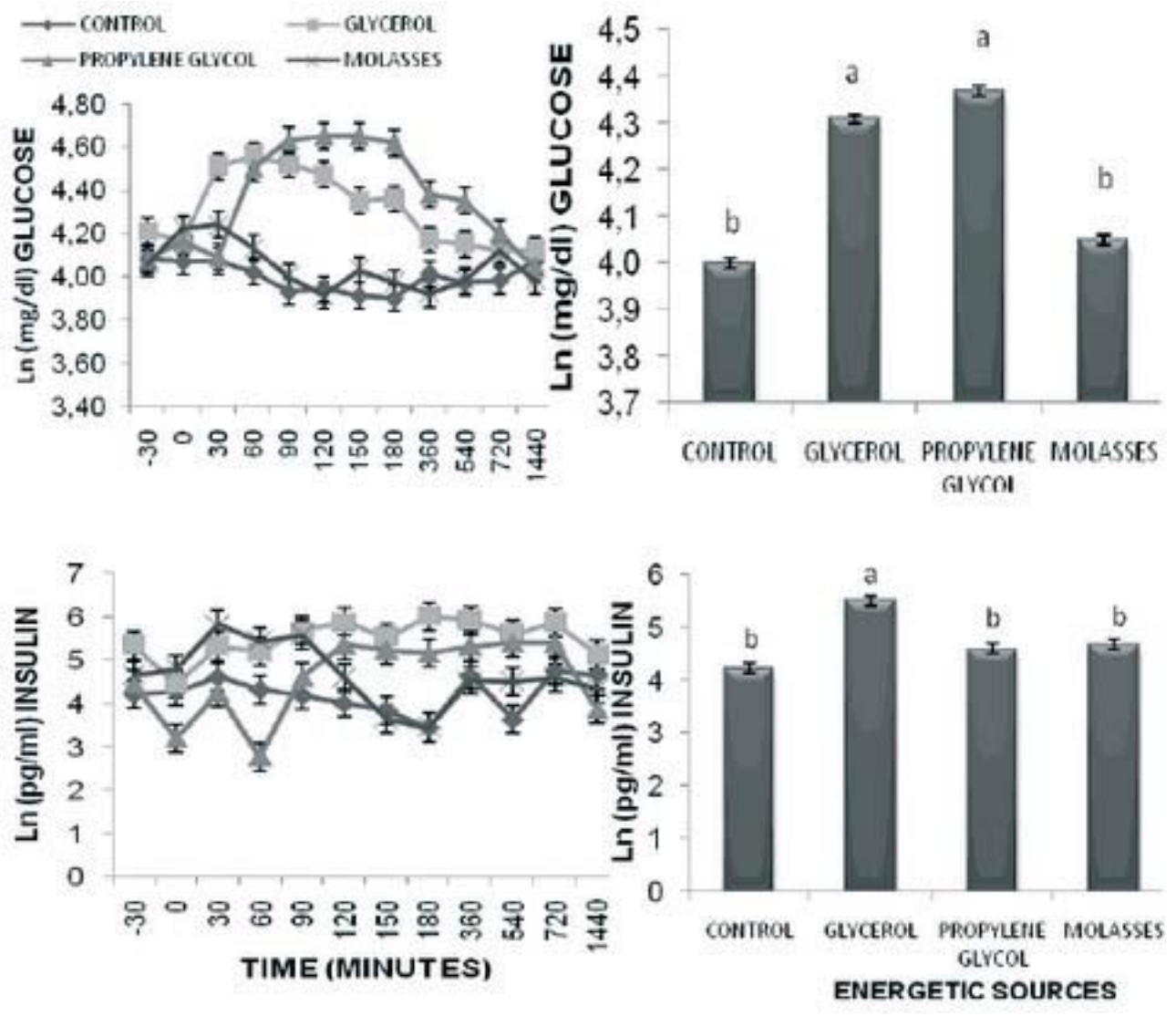

Figure 3. Mean blood concentrations of insulin and glucose at different times after an oral drench (1470.8 Kcal) of glycerol, propylene glycol or molasses in ewes. Values are means ± S.E.D. of logarithmic transformation of insulin and glucose concentrations. Mean values with different letters differ ($P < 0.05$).

gonadotropin responsive follicles. It appears that the increase of glucose and insulin is responsible for the increase in follicular development and, consequently, for the number of available follicles for ovulation. But then, could any nutritional of hormonal treatment that increases insulin cause an increase in ovulation rate

We conducted a series of studies to test the effect of treatments that enhance glucose redistribution on the number of small antral follicles growing in the ovaries. However, an increase in the number of gonadotropin responsive follicles is difficult to evaluate in sheep, as the diameter of the gonadotropin sensitive follicles (2 mm) is close to the lower range of sensitivity of the ultrasound scanner. To circumvent this inconvenience, we carried out the first study in cattle, where the gonadotropin sensitive follicles grow up to 4 mm in diameter and can be easily counted by transrectal ultrasound scanning. In addition, we used the gonadotropin inhibited model [16], where cattle are treated chronically with a GnRH agonist for a long period of time, with a consequent inhibition of the pulsatile release of LH and FSH to their basal concentrations, and the arrest of follicle development at 4 mm in diameter. Since dominant follicles do not develop, the gonadotropin sensitive follicles grow without the restrain and the fluctuating inhibition of dominant follicles.

Nineteen cows were treated with a continuous infusion of buserelin (37.5 ng/h) for six months after which

Table 4. Ovulation type and ovulation rate in Pelibuey ewes following ultrashort flushing (USF) with oral administration of 50, 100, 200 or 300 mL of a glycogenic solution (glycerol: water; 90:10 v/v) at the time of estrus synchronization with PGF2α.

Treatment Group	Ovulation type (%)				Ovulation rate
	Single	Double	Triple	Quadruple	
Control[a]	38.60	54.39	7.02	0	1.68 ± 0.05
50 mL[b]	10.71	78.57	10.71	0	2.00 ± 0.08
100 mL[b]	3.85	76.92	3.85	15.38	2.30 ± 0.15
200 mL[b]	14.29	60.71	14.29	10.71	2.21 ± 0.15
300 mL[b]	17.24	65.52	13.79	3.45	2.03 ± 0.12

[a,b]Different superscripts within a column differ ($P < 0.0001$).

the emergence of large follicles did not occur. Sequential treatments with glycerol (lt of a 90% glycerol:water solution) for 4 days, followed by a Beta-agonist receptor treatment (2.2g zilpaterol daily) for 4 days and a final treatment of a single bolus injection of recombinant growth hormone (BST, 500 mg) were given to all animals. Between treatment periods cows were left untreated for a period of a minimum of 5 days to avoid the carry over effect of one treatment to the next. The numbers of follicles in the ovaries were counted 4 days before treatment and 4 days after treatment, with exception of BST where the scanning was performed for 6 days after treatment.

Glycerol and zilpaterol increased ($P < 0.05$) the number of antral follicles 4 days after the start of treatment by 38 and 23% respectively (Table 5). In contrast, BST took longer to increase the number of antral follicles as at 4 days after treatment there were no changes in the number of follicles. However, by day 5 and 6 after treatment the number of follicles increased 27 and 45% respectively ($P < 0.05$) (Table 5). In all cases, the increase in follicle number was associated with an increase in glucose, insulin and IGF-I plasma concentrations.

We further tested if these treatments, when applied to sheep, would increase the number of follicles ovulating. Sheep were synchronized with progestin intravaginal sponges for 13 days and were simultaneously treated with a Beta-agonist (zilpaterol, 0.15 mg/kg/day) or served as control. There was no difference in food intake between experimental groups. Glucose and insulin blood concentrations were increased in sheep consuming the Beta-agonist. The ovulation rate was increased

Table 5. Follicle number in sheep before and after oral daily administration of either 300 mL of a glycogenic solution (glycerol:water; 90:10 v/v daily) for six days, 2.2 g of zilpaterol/cow/day for five days, or a single subcutaneous injection of slow-release recombinant bST.

Treatment group	Follicle Number			*P* value
	Pretreatment (n=19)		Post-treatment (n=19)	
Glycerol	25.4 ± 0.3	Day 4	35.12 ± 1.8	<0.05
Zilpaterol	31.2 ± 2	Day 4	38.5 ± 2	<0.05
	26.5 ± 2.8	Day 4	26.2	0.35
bST		Day 5	33.8	<0.05
		Day 6	37.4	<0.05

twofold from 1.37 in the control group to 1.72 in the treated group ($P < 0.01$) (Table 6) [7]. Fertility was similar in both groups, however the treatment with zilpaterol increased the proportion of ewes lambing twins as compared to the control ($P < 0.05$) (Table 7) [7].

As for growth hormone, studies by other researchers have shown that it increases the number of follicles growing in the ovaries, and that these follicles respond to exogenous gonadotropins by augmenting the response to superovulatory treatments in heifers [14,15]. If a similar increase in follicle number is achieved in sheep, the low strength of the dominant follicle may permit by itself an increase in the number of follicles and ovulations. We synchronized the estrous of 92 ewes and treated them with 125 mg of bST five days before the end of progestin synchronization. Treatment with bST increased

Table 6. Distribution of ovulation type in Pelibuey and F1 Pelibuey-Dorper ewes following administration of zilpaterol hydrochloride (0.15 mg/kg/day) for 13 days mixed in the feed from the start of estrous synchronization with FGA until mating.

Treatment Group	n	Ovulation type (%)		
		Single	Double	Triple
Control[a]	52	63	37	0
Zilpaterol[b]	50	34	60	6

[a,b]Different superscripts within a column differ ($P < 0.005$).

Table 7. Distribution of type of parturition in Pelibuey and F1 Pelibuey-Dorper ewes following administration of zilpaterol hydrochloride (0.15 mg/kg/day) for 13 days mixed in the feed from the start of estrous synchronization with FGA until mating.

Treatment Group	n	Type of parturition (%)	
		Single	Multiple
Control[a]	61	85	15
Zilpaterol[b]	68	71	29

[a,b]Different superscripts within a column differ ($P < 0.05$).

($P < 0.01$) the proportion of ewes lambing twins or triplets and the number of lambs born per ewe (Table 8) [4].

Taken together, the latter results show that treatment with (non-gonadotropin) hormones that affect nutrient partitioning may affect folliculogenesis and increase ovulation rate without changes in nutrient intake.

VII. CONCLUDING REMARKS

Nutrition enhances ovulation rate in sheep by either an increase in body weight (static effect), or by changes in metabolic hormones (acute effect). Both of these effects enhance the ovulation rate by increasing the number of gonadotropin responsive follicles. Herein, we described a series of studies that demonstrate a hyper-acute effect of nutrition that works at the time of luteolysis to select supplementary follicles for ovulation. The hyper-acute effect of nutrition seems to work by the already proposed [33] reduction in estradiol production as the abundance of mRNA for P450 aromatase declined 12 h after the glycogenic drench. These studies also show that ovine follicles can develop ovulatory capacity within 48 h after being selected when coinciding with the follicular phase.

We have developed an ultrashort flushing method with glycogenic solutions that could be administered together with other managements (*e.g.* internal deworming) and that will increase the ovulation rate in the flock. However, we believe that not all glycogenic substances will work equally and that only those that increase insulin concentrations have the capacity to

Table 8. Proportion of ewes lambing singletons, twins or triplets from ewes treated or not with bST (125 mg) five days before progestin sponge withdrawal.

Groups	n	Percent of ewes lambing			Lambs born per ewe lambing
		Singletons	Twins	Triplets	
bST	47	43.5	48.7	7.7	1.64
Control	45	74.3	25.7	0	1.25

The lambing pattern differ between groups ($P < 0.01$).

augment the number of ovulations, although this remains to be tested.

Finally, hormonal flushing can be achieved without changes in the energy intake of the sheep. Both BST and Zilpaterol increased ovulation rate and prolificacy and could be adapted as a management practice to increase the profitability of the farm.

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