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Ovulation rate, prolificacy and pregnancy rate in goats treated with oral glycerol

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Abstract

This study tested whether the oral administration of glycerol at the time of progestin removal and in the first 6 days of the estrous cycle increased the ovulation rate, prolificacy, and pregnancy rate in goats. Intravaginal sponges impregnated with fluorogestone acetate were inserted into 129 goats for 12 days; upon sponge removal, goats were randomly assigned to one of the two following treatment groups: the glycerol group (n = 65), which received an oral drench of 100 mL of glycerol upon sponge removal and was repeated on days 0, 2, 4, and 6 following estrus (estrus = day 0), and the control group (n = 64), which did not receive glycerol. The goats in estrus were mated, and their ovulation rate was determined by ultrasonography between days 8 and 12 of the estrous cycle. Pregnancy was diagnosed by ultrasonography on day 40, and the prolificacy was determined at birth. In 6 goats treated with glycerol and 5 controls, their insulin concentrations at 0, 2, 4, 8, and 12 h after the glycerol drench were determined by radioimmunoassay. The proportion of goats with multiple ovulations (glycerol = 71 vs control = 64) and the proportion of goats with multiple births (glycerol = 52 vs control = 56) were similar ($P > 0.05$) between treatments. Likewise, the pregnancy rate was similar ($P > 0.05$) between treatments (glycerol = 88 vs control = 85%). The insulin concentrations tended to be higher in goats treated with glycerol ($P = 0.08$). In conclusion, an oral drench of 100 mL of glycerol at the time of progestin withdrawal and in the first 6 days of the estrous cycle did not increase the ovulation rate, prolificacy, or pregnancy rate in goats.

Keywords: Ovulation rate; Prolificacy; Glycerol; Insulin; Goats.

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Introduction

An increase in the ovulation rate and prolificacy in small ruminants has been achieved in an efficient way through hormone treatments and by increasing the energy content in the diet (flushing). Although the flushing mechanism is not yet fully understood, the evidence indicates that its effects are produced at the ovarian level; furthermore, they are independent of gonadotropin concentrations and are associated with increases in the blood glucose and insulin levels (Downing *et al.*, 1995; Muñoz-Gutiérrez *et al.*, 2004; Dupont *et al.*, 2014).

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Vol. 3 | No. 1 | January-March | 2016

In sheep, short-term supplementation with lupin grain, a legume with a high metabolizable energy and protein content, can increase ovulation rates. Short-term supplementation with lupin grain stimulates folliculogenesis and increases ovulation rates, without inducing changes in body weight and condition (Scaramuzzi *et al.*, 2006). Glucose and insulin may influence the ovulation rate by stimulating follicle development, resulting in a higher number of follicles capable of responding to gonadotropin stimulus (Gutiérrez *et al.*, 1997; Viñoles *et al.*, 2005). Furthermore, glucose and glucosamine stimulate follicle steroidogenic capacity by increasing insulin-like growth factor type I (IGF-I) activity (Muñoz-Gutiérrez *et al.*, 2004). Studies performed in goats show that an increase in insulin, either induced by diet or by insulin administration, increases the ovulation rate (Suguna *et al.*, 2009; Zabuli *et al.*, 2010).

The administration of glycogenic solutions can cause an increase in the blood glucose and insulin concentrations. In sheep, short-term treatments with glycerol and propylene glycol have been used to increase ovulation rates. Rodriguez Iglesias *et al.* (1996) were able to increase the ovulation rate in seasonally anovulatory ewes through the oral administration of 100 mL of a glycerol and propylene glycol solution, which were administered immediately before ram exposure. Gutierrez *et al.* (2011) observed increased serum glucose concentrations, insulin, and ovulation rates with a single oral dose of 300 mL of glycerol during the induction of luteolysis with PGF2α in Pelibuey sheep. In the same study, a similar effect on the ovulation rate with a single oral dose of 100 mL of glycerol was achieved.

Moreover, insulin promotes early embryonic development. *In vitro* insulin exerts a mitogenic and an antiapoptotic effect on embryos (Byrne *et al.*, 2002; Augustin *et al.*, 2003), resulting in an increased proportion of embryos reaching the blastocyst stage. Additionally, high insulin concentrations negatively affect embryonic development in cattle (Fouladi-Nashta *et al.*, 2006) and sheep (Carrera-Chávez *et al.*, 2014). Furthermore, a positive effect from insulin on luteal function has been observed. In a goat study, insulin was associated with higher concentrations of progesterone during pregnancy (Suguna *et al.*, 2009). High levels of progesterone are associated with accelerated embryonic development (Garret *et al.*, 1988), which could translate into fewer embryonic losses. In dairy cows, the administration of 1 L of oral glycerol during the first 6 days post-insemination increases blood insulin levels and increases the pregnancy rate (Ortega *et al.*, 2010). Therefore, this study tested whether the oral administration of 100 mL of glycerol, at the time of progestin removal and in the first 6 days of the estrous cycle, increased the ovulation rate, prolificacy, and pregnancy rate in goats.

Materials and methods

Location

This study was conducted in an experimental station at the Facultad de Medicina Veterinaria y Zootecnia of Universidad Nacional Autónoma de México (UNAM), located in Tequisquiapan, Querétaro, at a latitude of 20°31'21" N and at 1881 m above sea level. The regional climate is temperate and semi-arid, with an average temperature of 17.5° C and an average annual rainfall of 512 mm (García, 1981). The experiment was carried out from September to November, which corresponds to the reproductive season of goats in Mexico (Arvizu *et al.*, 1995).

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Vol. 3 | No. 1 | January-March | 2016

Animals

In this experiment, 129 crossbreed (Boer-Alpine French) cycling goats (22 doelings and 107 does varying in parity) with a body condition score between 2 and 3 were included (Russel *et al.*, 1969). The goats were confined and fed a diet based on alfalfa hay, corn silage, and concentrate according to NRC requirements. This experiment was approved by the Consejo Académico del Posgrado en Ciencias de la Producción y de la Salud Animal from UNAM.

Treatments

In all goats, an intravaginal sponge impregnated with 45 mg of fluorogestone acetate (FGA; Chronogest; MSD Animal Health, Mexico; Intervet México, S.A. de C.V., Huixquilucan, Estado de México, México) was inserted and remained *in situ* for 12 days. When the sponge was removed, a luteolytic dose of sodium cloprostenol (Celosil; MSD Animal Health, Mexico) was administered. Upon sponge withdrawal, the goats were randomly assigned to one of the following treatment groups: the glycerol (n = 65) group, which received an oral drench of 100 mL of glycerol repeated on days 0, 2, 4, and 6 following estrus (estrus = day 0), and the control (n = 64) group, which did not receive glycerol. Males fitted with an apron performed estrus detection. Females in estrus were mated to males of proven fertility.

Between days 8 and 12 post-mating, the ovulation rate was determined via a transrectal ultrasound exam by counting the number of corpora lutea; for this purpose, a 7.5 MHz linear transducer (Aloka, Co., Ltd., Tokyo, Japan) was used, which was adapted to a rigid support to allow for handling (Gutierrez *et al.*, 2011). The pregnancy rate was determined by ultrasonographic pregnancy diagnosis on day 40 after service. Prolificacy was determined at birth (Fig. 1).

Blood sampling and sample processing

To determine blood levels of progesterone and insulin, 6 goats were treated with oral glycerol and 5 controls were randomly sampled. Blood samples for progesterone quantification were taken daily between days 1 and 17 of the estrous cycle. Two goats in the control group presented with short cycles; therefore, they were not included in the progesterone statistical analysis. On day 4 of the estrous cycle, sampling was conducted in order to determine blood insulin levels induced by the oral administration of glycerol. The first sample was taken before treatment (0 h), and the next samples were taken at 2, 4, 8, and 12 h after the glycerol drench.

Blood samples (10 mL) were obtained by puncture of the jugular vein using Vacutainer® tubes containing 100 µL of sodium citrate. The blood samples were centrifuged at 1500 x g for 10 min to separate the plasma, which was subsequently stored at -20 °C until analysis. The progesterone and insulin concentrations were determined by solid-phase radioimmunoassay (Coat-A-Count®, RIA kit, DPC; USA). In the case of progesterone, the assay sensitivity was 0.1 ng/mL with an intra-assay variation coefficient of 4.2%, while the insulin sensitivity was 0.052 ng/mL, with an intra-assay variation coefficient of 3.3%.

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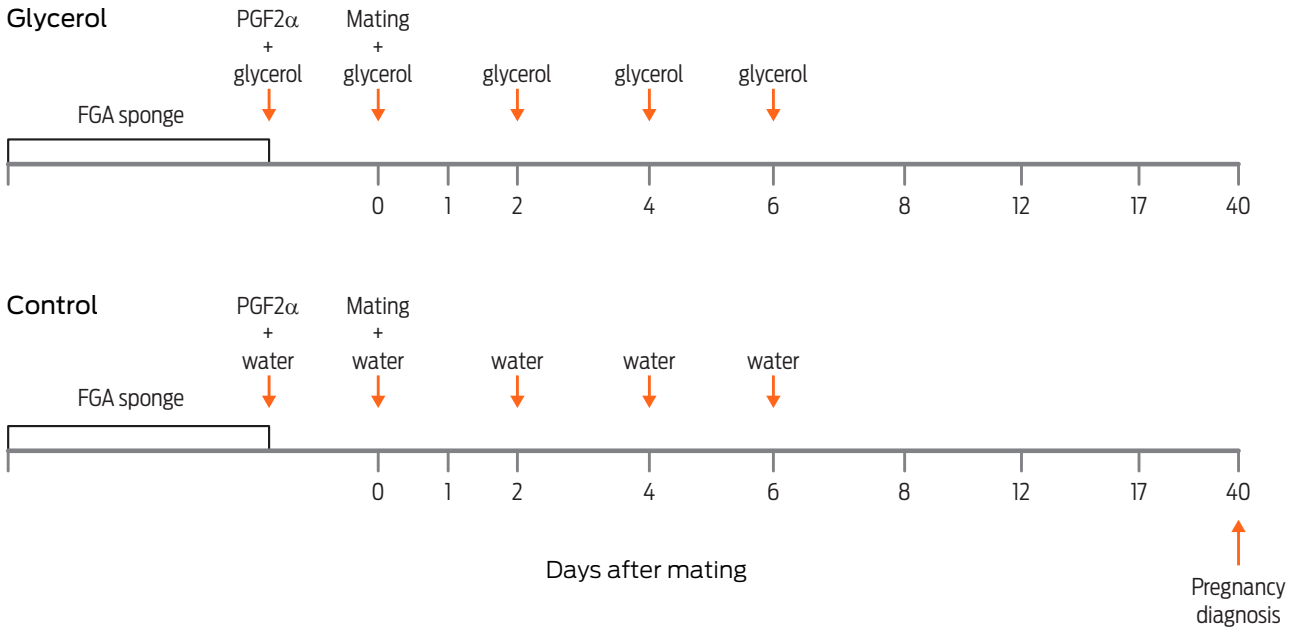

Figure 1. A schematic overview of the treatments.

Statistical analysis

A logistic regression model was used to determine the effect of oral glycerol treatment on the percentage of goats with multiple ovulations and multiple births. Likewise, the effect of parity (primiparous and multiparous) and its interaction with treatment was tested by logistic regression [SAS version 9.2 (SAS Institute Inc., Cary, NC)]. The ovulation rate and prolificacy were compared by the Mann-Whitney U test, in which the effect of treatment and its interaction with parity were considered. The differences in the progesterone and insulin concentrations between treatments were tested by ANOVA for repeated measurements; the model included treatment, time, and their interactions. In all cases, $P < 0.05$ was considered to be significantly different and $P \leq 0.10$ was considered to be a tendency.

Results and Discussion

The oral administration of 100 mL of glycerol at the time of progesterone withdrawal did not increase the ovulation rate, and the proportion of goats with multiple ovulations or multiple births was similar between treatments (Tables 1 and 2). The ovulation rate [glycerol= 1.84 ± 0.64 vs control=1.80 ±0.72 (mean ± standard deviation)] and the prolificacy [glycerol= 1.58 ± 0.61 vs control=1.60 ±0.64 (mean ± standard deviation)] were similar between treatments. These results are different from those observed in sheep by Rodriguez Iglesias *et al.* (1996) and Gutierrez *et al.* (2011). The reason the treatment did not have a favorable effect on the ovulation rate may be related to the metabolic response to the oral glycerol doses. The 100 mL glycerol dose was determined based on the results obtained in sheep by Gutierrez *et al.* (2011); in their study, the administration of 100 mL glycerol caused an increase in the ovulation rate similar to the one obtained with 300 mL. For this reason, the administration of 100 mL was chosen. In the present study, serum

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Vol. 3 | No. 1 | January-March | 2016

Table 1. Percentage of goats with multiple ovulations that received an oral drench of 100 mL glycerol.

Variable	Class	Percentage of multiple ovulation	Odds ratio	95% confidence interval	P-value
Treatment	Control	64 (29/45)	1.00		
	Glycerol	71 (35/49)	1.73	0.66-4.49	0.260
Parity	Primiparous	31 (5/16)	1.00		
	Multiparous	75 (59/78)	7.56	2.25-25.31	0.001

Table 2. Percentage of goats kidding more than one kid that received an oral drench of 100 mL glycerol.

Variable	Class	Percentage of multiple births	Odds ratio	95% confidence interval	P-value
Treatment	Control	56 (26/46)	1.00		
	Glycerol	52 (25/48)	0.95	0.40-2.20	0.906
Parity	Primiparous	27 (4/15)	1.00		
	Multiparous	59 (47/79)	4.00	1.16-13.80	0.002

insulin concentrations were not affected by the treatment (P = 0.11), but there was an interaction between the treatment and time (P = 0.08). Thus, insulin concentrations tended to be higher within 2 and 4 h post-treatment (Fig. 2). Likely, this slight increase in insulin concentrations was not enough to improve the ovulation rate. The cause of the variation in response to the same glycerol dose observed between sheep and goats is unknown. Studies evaluating glycogenic solutions have been performed in sheep and cattle, but not in goats (Rodriguez-Iglesias *et al.*, 1996; Ortega *et al.*, 2010; Gutierrez *et al.*, 2011). Although these 3 species are ruminants, they present differences in their digestive function and their use of forages. Goats have physiological adaptations that allow them to leverage high fiber feeds more efficiently (Silanikove, 2000). Therefore, they may be less sensitive to variations in the quality and quantity of offered feeds. Oral glycerol is used as a substrate in rumen fermentation from which propionate can be obtained. However, it can also be absorbed directly as glycerol through the rumen mucosa, which will be converted later into glucose in the liver (Trabue *et al.*, 2007). Although this metabolic pathway is the same in ruminants, its efficiency in triggering insulin secretion may be different between species; this could explain, in part, the success of the treatment in increasing the ovulation rate in sheep as well as its failure in goats. The results of this study promote the development of future studies in goats to evaluate different glycerol doses, until the dose that triggers an optimal response in serum insulin is determined.

Furthermore, this study proposed that the oral administration of glycerol during the first days of embryo development (days 0, 2, 4, and 6 of the estrous cycle) favors embryonic survival, which would increase the proportion of pregnant goats and

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Vol. 3 | No. 1 | January-March | 2016

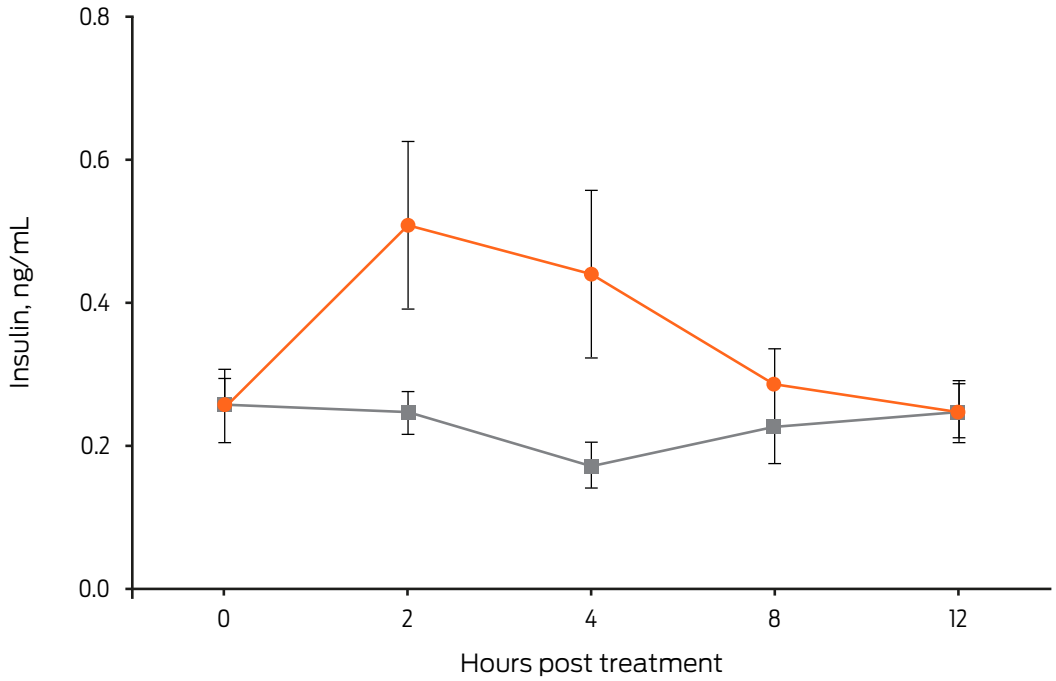

Figure 2. Average insulin concentrations (± standard error) in goats treated with 100 mL of glycerol (—●—) and controls (—■—). Treatment by time interaction: P = 0.08.

prolificacy. This hypothesis was based on the expected effect of glycerol on insulin concentrations and the effects of insulin on embryonic development. Studies performed *in vitro* and *in vivo* show that insulin improves embryonic development by increasing cell proliferation and decreasing apoptosis (Augustin *et al.*, 2003; Sugu-na *et al.*, 2009). Meanwhile, low insulin concentrations adversely affect embryonic development and interferon tau production (Thatcher *et al.*, 1995). Furthermore, in dairy cows, an increase in insulin concentrations induced by the oral administration of 1 L of glycerol during the first 6 days of embryonic development (days 0, 2, 4, and 6) has been shown to improve pregnancy rates (Ortega *et al.*, 2010). In the present study, however, the pregnancy rate (glycerol = 88 vs control = 85%) and the proportion of multiple births were similar between treatments ($P > 0.05$; Table 2). The lack of effect on prolificacy and pregnancy rate observed in this study was probably due to the marginal increase in insulin concentrations after the oral administration of 100 mL of glycerol. Furthermore, the pregnancy rate obtained in both groups was high, which makes it more difficult to identify a positive effect from any treatment aimed at improving fertility. Parity was observed to have an effect on ovulation rate and prolificacy; however, there was no interaction between the treatment and parity (Tables 1 and 2; $P > 0.1$).

The administration of oral glycerol during the first days of the estrous cycle may favor embryonic development and conception rate by increasing serum progesterone concentrations. This hypothesis is based on studies in cattle in which insulin increases circulating progesterone concentrations (Spicer y Echternkamp, 1995; Cooke *et al.*, 2012). In this study, nonetheless, progesterone concentrations between 1 and 17 days post-mating were similar between treatments, and no interaction was observed between the treatment and time ($P > 0.05$; Fig. 3).

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Vol. 3 | No. 1 | January-March | 2016

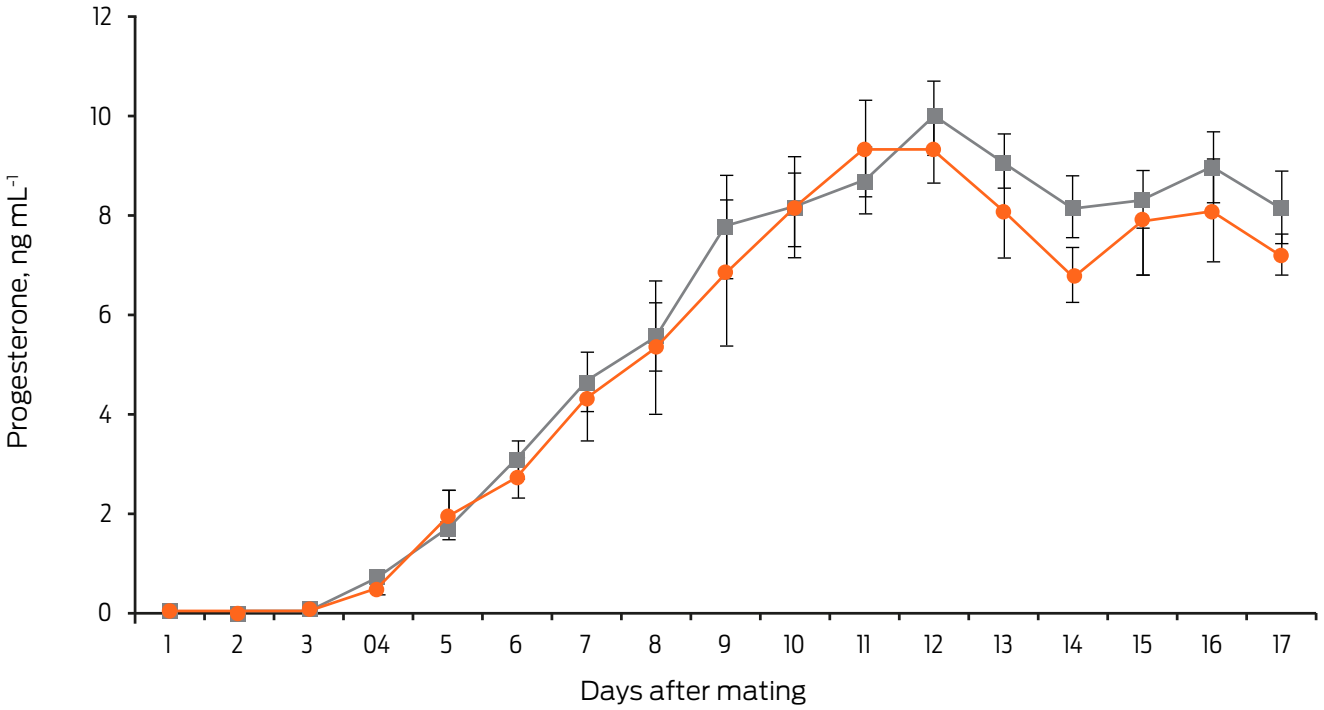

Figure 3. Average concentrations of progesterone (± standard error) in goats treated with 100 mL of oral glycerol (–■–) and controls (–●–) ($P > 0.05$).

The good body condition of the goats used in this study, as well as the season in which the treatment was applied, may have influenced the lack of a positive response to glycerol administration. The goats received a diet that met the NRC requirements, and they had a body condition between 2 and 3 at the beginning of the experiment, which is reflective of the conditions associated with a high pregnancy rate and high prolificacy and are similar to those obtained consistently within the flock. Conversely, this study was completed during the height of the reproductive period, which contributed to the excellent response in fertility. Future experiments should be conducted in conditions in which the fertility and prolificacy are diminished, such as in cyclicity induction programs during anestrus or in marginally nourished goats; in these circumstances, the supplementation proposed in this study may improve fertility. Treatments exist that do not increase pregnancy rates globally, but rather do so in subgroups of animals. In a previous study conducted by our group, for example, bovine recombinant somatotropin only increased pregnancy rate in goats in anestrus, as well as only increased prolificacy in primiparous goats, who naturally tend to have a lower pregnancy rate and prolificacy (Hernández y Gutiérrez, 2012).

Conclusions

In conclusion, the oral administration of 100 mL of glycerol at the time of progestin withdrawal and in the first six days of the estrous cycle did not increase the ovulation rate, prolificacy, or pregnancy rate in goats.

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Vol. 3 | No. 1 | January-March | 2016

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Ubaldo Aguilar performed the experiment and analyzed the results.

Yesmín Domínguez performed the experiment.

Joel Hernández Cerón and Carlos G. Gutiérrez designed the experiment, analyzed the results and wrote the manuscript.

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Vol. 3 | No. 1 | January-March | 2016

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