

# EFFECT OF PGF<sub>2</sub> $\alpha$ ADMINISTRATION BEFORE UTERINE FLUSHING ON EMBRYO RECOVERY RATE IN SUPEROVULATED COWS AND HEIFERS

## Efecto de la administración de PGF2 alfa antes del lavado uterino sobre la tasa de recuperación embrionaria de vacas y vaquillas superovuladas

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### ABSTRACT

Donor cows usually receive an injection of PGF<sub>2</sub> alpha or analogues (PG) after embryo recovery to avoid occurrence of pregnancy. Due to its capacity to stimulate uterine contractions it has been suggested that an increase in embryo recovery could be obtained if the PG is injected before collection. The aim of this experiment was to evaluate, if the injection of PG 3 h before collection improves embryo recovery. A group of 334 donor cows and heifers (302 Holstein, 28 Brown Swiss and 4 Jersey), were superovulated with FSH-P, inseminated and assigned according to breed and parity to the following groups: (1) treatment group (114 heifers and 31 cows) were injected with 50 mg of PGF<sub>2</sub> alpha, 3 hours before embryo collection and (2) control group (121 heifers and 38 cows) were injected with saline at the same time interval. Embryos were recovered non-surgically on days 6.5 to 7.5 following estrus. Number of corpora lutea, recovery rate of embryos and number of transferable embryos in each group were recorded. No differences (P>0.05) were found for any parameter between the treated and the control groups. The results show that the injection of PG 3 hours before embryo collection does not improve embryo recovery rate.

**Key words:** Embryo recovery, prostaglandin F2 alpha, bovine.

### RESUMEN

Las vacas donantes de embriones usualmente reciben una inyección de PGF<sub>2</sub> alfa o sus análogos (PG) después de la colección de embriones, para evitar que ocurra la gestación. Debido a su capacidad para estimular las contracciones uterinas,

se ha sugerido que la recuperación embrionaria pudiera ser aumentada, si se inyecta PG antes de la colecta. El objetivo del presente experimento fue el de evaluar si la inyección de PGF<sub>2</sub> alfa 3h antes de la colecta mejora la tasa de la recuperación embrionaria. Se utilizaron 334 vacas y vaquillas donantes (302 Holstein, 28 Pardo Suizo y 4 Jersey), las cuales fueron superovuladas, inseminadas y asignadas de acuerdo a la raza y paridad a los siguientes grupos: (1) o tratado (114 vaquillas y 31 vacas) que fueron inyectadas con 50 mg de PGF<sub>2</sub> alfa, 3 h antes de la colección embrionaria y 2) el grupo testigo (121 vaquillas y 38 vacas) que se inyectaron con solución salina al mismo intervalo. La colección no quirúrgica se hizo 6,5 a 7,5 días postestro. Se evaluó el número de cuerpos lúteos, la tasa de recuperación y el número de embriones transferibles en cada grupo. No existió diferencia (P>0,05) en ninguno de los parámetros entre el grupo tratado y el testigo. Los resultados muestran que la inyección de PFG<sub>2</sub> alfa 3 h después de la colecta no mejoró la tasa de recolección embrionaria.

**Palabras clave:** Recolectión embrionaria, prostaglandina F2 alfa, bovino.

### INTRODUCTION

In embryo transfer procedures it frequently occurs that the number of ova/embryos found after flushing is usually less than the number of corpus luteum (CL) present in the ovaries of the female donor. Thus a certain proportion of embryos are not recovered, and the mean recovery rate varies from 65.1 to 70.1% [21].

Superovulatory treatments impaired transport of gametes and zygotes, due to changes in steroid hormones production [2, 9]. Studies on ova transport in superovulated cows indi-

cates that 56% of the ova entered the uterus between 48 and 72 h after ovulation [17] and most of them (92%) are in the uterus by day 7 in which collection normally will be performed, but 8% are still in the oviducts by this time [19] and would not be recovered by non-surgical flushes of live animals [9].

Since PGF2 $\alpha$  or its analogue preparations (PG) stimulates oviductal [30] and myometrial contractility [24], due to its spasmogenic properties it has been proposed that treatment with PG before embryo collection could contribute to increase the number of embryos recovered.

There is evidence, that in human [30] and Hamster [33] PGF2 $\alpha$  accelerate zygote transport in the oviduct, when given in large pharmacologic doses.

Therefore, investigations of drugs that promote transport in the oviduct and contribute to increase embryo recovery rate are of relevant importance.

Normally, donors are treated with prostaglandins after flushing in order to induce lysis of the corpora lutea and return to estrus, thus avoiding pregnancy [1, 6, 8]. The treatment with prostaglandin before embryo collection could exert a beneficial effect without extra expense.

The aim of this study was to investigate the effect of PGF2 $\alpha$  on embryo recovery rates in a commercial embryo transfer program.

## MATERIALS AND METHODS

This study was conducted in an Embryo Transfer Center located in the state of Mexico, Mexico. A total of 334 donors (64 cows and 265 heifers) of different dairy breeds (302 Holstein, 28 Brown Swiss and 4 Jersey) were employed. Heifers were between 16 and 20 months old. Lactating cows with different parity (2 to 8 parturition) and more than 80 d after calving were used. Some cows (78%) had been submitted previously to superovulation.

The females were clinically normal, with regular estrous cycles. Animals were assigned according to breed and parity to two groups: Group 1 or experimental (144 heifers and 31 cows) received 50 mg of PGF2 alpha (Lutalyse, Upjhon, México) 3 h before flushing and Group 2 or control (121 heifers and 38 cows) that were treated with saline at the same time interval. An interval of 3 h from PG injection to flushing was selected in order to allow the prostaglandin to perfuse and exert his action [24].

All donors were superovulated between days 9 and 11 after estrus, with 24 to 36 mg of FSH-P (Scheramex, México) in a decreasing 4 day schedule [20]. On the third day they were injected with 25 mg of PG in the morning and in the evening (08:00 and 20:00h, respectively) [13], and were observed every two hours until standing heat. All donors were inseminated 12 and 24 h after onset of estrus with frozen-thawed se-

men from proven fertile bulls. On day 6.5 - 7.5 following estrus, donor females were collected non-surgically and corpora lutea number was determined by rectal palpation.

Late morula and blastocyst were evaluated and considered as transferable embryos using standard morphological criteria according to their development stage and quality [11, 33].

Recovery rate was calculated by dividing the number of total ova/embryos by the number of CL.

The data in the TABLES are expressed as mean $\pm$ SE. A one way analysis of variance (ANOVA) was applied to compare the number of corpora lutea, total ova and transferable embryos between groups using the statistical analysis system (SAS) [26]. Usual assumption of ANOVA were tested and the hypothesis of samples normally distributed with homogeneous variance were not rejected.

Data expressed as a proportion or percentage were evaluated by Chi-square analysis.

## RESULTS

There were no differences between groups in the number of corpora lutea as determined by routine examination of the ovaries (TABLE I). Heifers had significantly more corpora lutea and transferable embryos than cows ( $P < 0.05$ ) (TABLE II).

To test if the number of embryos obtained was affected by the collection, the efficiency of embryo recovery was esti-

TABLE I  
NUMBERS OF CORPORA LUTEA, TOTAL OVA AND TRANSFERABLE EMBRYOS IN ANIMALS INJECTED WITH PGF2 ALPHA 3 HOURS BEFORE EMBRYO COLLECTION AND IN THE CONTROL GROUP

|                             | n   | Experimental group<br>(X $\pm$ SEM) | n   | Control group<br>X $\pm$ SEM |
|-----------------------------|-----|-------------------------------------|-----|------------------------------|
| <i>Corpora lutea</i>        |     |                                     |     |                              |
| Heifers                     | 144 | 11.2 $\pm$ 0.6 <sup>a</sup>         | 121 | 11.8 $\pm$ 0.7 <sup>a</sup>  |
| Cows                        | 31  | 8.1 $\pm$ 1.0 <sup>b</sup>          | 38  | 9.4 $\pm$ 1.1 <sup>b</sup>   |
| <i>Ova recovered</i>        |     |                                     |     |                              |
| Heifers                     | 144 | 8.1 $\pm$ 0.5 <sup>a</sup>          | 121 | 7.9 $\pm$ 0.6 <sup>a</sup>   |
| Cows                        | 31  | 6.4 $\pm$ 1.1 <sup>b</sup>          | 38  | 7.4 $\pm$ 1.0 <sup>a</sup>   |
| <i>Transferable embryos</i> |     |                                     |     |                              |
| Heifers                     | 144 | 5.6 $\pm$ 0.4 <sup>a</sup>          | 121 | 5.0 $\pm$ 0.4 <sup>a</sup>   |
| Cows                        | 31  | 3.8 $\pm$ 0.9 <sup>b</sup>          | 38  | 3.9 $\pm$ 0.7 <sup>b</sup>   |

Within rows and columns, means without a common superscript are different ( $P < 0.05$ ).

**TABLE II**  
**RECOVERY RATE IN DONORS INJECTED WITH PGF2**  
**ALPHA BEFORE EMBRYO COLLECTION AND**  
**IN THE CONTROL GROUP**

|         | n   | Experimental group (%) | n   | Control group (%) |
|---------|-----|------------------------|-----|-------------------|
| Heifers | 144 | 68.2 <sup>a</sup>      | 121 | 67.4 <sup>a</sup> |
| Cows    | 31  | 71.1 <sup>b</sup>      | 38  | 74 <sup>b</sup>   |

Within rows and columns, means without a common superscript are different ( $P < 0.05$ ). Chi-square.

mated as number of collected structures (ova/embryos) divided by the number of CI present in the ovaries. Prostaglandin administration did not affect the percentage of embryo collected ( $P > 0.05$ ). Nonetheless, recovery in cows was significantly higher ( $P < 0.05$ ) than in heifers (TABLE II).

No differences were found between groups in the recovery rate ( $P > 0.05$ ) (TABLE II).

## DISCUSSION

The present data show that the treatment with PG before flushing does not improve embryo recovery rate in heifers and cows.

Rodriguez-Martinez et al [22, 23, 24] studied uterine and oviductal contractility in response to PGF2 alpha. They found and immediate significant increase in base line tone of uterine contraction following treatment with PGF2 alpha. Spontaneous uterine activity patterns occurred or resumed within 20 min after treatment in all cows [24].

Similarly, treatment with PGF2 alpha increase muscular activity in vivo and in vitro of pig oviducts, with a duration of the drug-effect of 10 to 20 min [23].

In this study, it may have occurred that the contraction caused by PGF2 alpha administration did not resemble the natural waves of motility of the myosalphynx, activity which is considered to be the most important factor for the transport of embryos to the uterus. Therefore, treatment with PG before embryo retrieval failed to promote the transport into the uterus of the embryos remaining in the oviducts and to increase embryo recovery rate.

The effect of PGF2 alpha is not likely to be mediated by the lack of progesterone, since concentration in this hormone dropped to below 1ng/mL by 30 h after PGF2 alpha injection in cows [18].

It is not known if the selected interval of 3 h from PG-injection to the embryo collection was correctly chosen since no information is available in cows, about the duration of the effect of PG injection during the early luteal phase on the oviductal transport of the zygotes. An interval of 3 h from PG in-

jection to flushing was applied in order to allow the prostaglandin to perfuse and exert his action.

A longer interval was not selected because the decrease in progesterone levels during the early luteal phase could compromise embryo recovery. Premature luteal regression is characterized by a decline of progesterone to basal levels 3-6 d after estrus [25]. It frequently occurred in the superovulated goat [3, 4, 5, 25] and sheep [27] and result in reduced embryo recovery and quality.

Heifers had significantly more corpora lutea and produced more transferable embryos than cows probably because the majority of the previously, and it is known that repeated superovulation decrease the response [7]. Advanced age of donors could be also a cause of the decrease in the number of transferable embryos found in cows [13].

In this study the efficiency of embryo recovery was calculated with relation to the number of CI detected at rectal palpation. It was recognize that there might be inaccuracies in CI estimation, but when ultrasound examination was performed it was not possible to differentiate the limits between adjacent corpus luteum. In contrast, rectal palpation allow to count the number of CI by their protuberances thus been a more reliable estimator of CI number.

Even though flushing and the searching for ova were performed by and experienced team, the recovery rate varied from 68.2 to 74%, which indicates that one third and one quarter of the total ova were not found, although they are in the range reported by other authors [21, 28].

There is little information about the causes of losses in ova/embryo. It has been reported that the increase in the ovarian size is inversely related to the number of ova/embryos recovered, due to reduce ability of the fimbriae to pick-up ovulated oocytes. These losses are therefore inevitable since hyperthrophy of the ovaries is a consequence of the gonadotrophin stimulation [29, 32, 34].

It is also possible that some oocytes remain in the luteinized follicles and never reach the oviducts [16].

Some authors have proposed that some embryos remain in the oviducts at the time of the flushing [10]. In beef heifers 6% of the ova/embryos have been found in the oviducts on day 7, but this accounted only for 1.2% of total recovery [15]. Impaired ova transport through the oviducts, accelerated or retarded, can be provoked by endocrine imbalances associated with superovulation and could therefore explain part of the losses [2, 14].

Finally, although non-surgical collection of embryos has been improved in the last decade, it has been shown that more ova/embryos can be recovered from the excised uteri of donors than from *in vivo* flushes [12], which means that the *in vivo* flushing procedure may be a cause of variation in embryo total ova recovery.

In conclusion, the results from this study show that the injection of PG before flushing does not improved embryo recovery.

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