

EFFECT OF PHENYLBUTAZONE ON PREGNANCY RATES OF EMBRYO RECIPIENT MARES

EFEITO DA FENILBUTAZONA SOBRE AS TAXAS DE PREENHEZ EM ÉGUAS RECEPTORAS DE EMBRIÕES

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ABSTRACT: This experiment evaluated the pregnancy rates of equine embryo recipients with different degrees of uterine tone (absent, fair moderate, intense), treated with phenylbutazone (PBZ). Thirty-two Paint Horse breed donors and 96 Mangalarga recipients were used during 2001/2002 breeding season (October to January). The embryos were collected 8 days after ovulation, classified and then transferred non-surgically. At the time of transfer, the recipients were divided into groups A to E according to uterine tone. Group A (n = 19) consisted of recipients with absent or fair uterine tone, which were not treated and received only the embryo; Group B (n = 14) consisted of recipients with absent or fair uterine tone, treated with 1 dose of 2 g PBZ; Group C (n = 11) consisted of recipients with absent or fair uterine tone, treated with 3 doses of 2 g PBZ, repeated at eight hour intervals; Group D (n = 23) consisted of recipients with moderate or intense uterine tone, treated with 1 dose of 2 g PBZ; Group E (n = 29) consisted of recipients with moderate or intense uterine tone, which were not treated and received only the embryo. At day 35 post embryo transfer the total pregnancy rate was 63.5%, being 36.8% (07/19); 35.7% (05/14); 63.6% (07/11); 82.6% (19/23) and 79.3% (23/29) for Groups A, B, C, D and E, respectively. The pregnancy rate for Group C was not significantly different from those observed in Groups D and E, but was greater ($P < 0.05$) than those observed in Groups A and B, indicating that three repeated doses of PBZ every 8 hours may be beneficial to recipients with poor uterine tone.

UNITERMS: Phenylbutazone; Embryo Transfer; Recipient; Mares.

INTRODUCTION

Progesterone is one of the hormones that controls the reproductive functions of mares and is essential for the beginning and development of pregnancy. Plasma progesterone concentrations increase after ovulation, reach a peak between day 5 and 7 and maintain stable levels up to day 13 or 14 (BRUCK et al., 1997). Good cervix and uterine tonicity at 5 days post ovulation is a clinical sign used to evaluate recipient mares (EAST et al., 1998). The best candidates are considered to be those with moderate to intense uterine tonus at the time of embryo transfer. In the Reproduction Laboratory of the Colorado State University (Fort Collins, CO), McCue et al. (1999), observed at day 5 lower serum progesterone levels in disqualified mares (6.5 ng/mL) compared to approved mares (10.0 ng/mL), and related low circulating progesterone levels to reduced uterine tonus. In a later report from the same Laboratory, Carnevale et al., (2000) detected a significantly lower pregnancy rate at 50 days among recipient mares with weak uterine tonus compared to those considered to have excellent tonus (50.9%; 84/165 versus 61.4%; 234/381).

In the nonsurgical method of embryo transfer, the

trauma caused by the passage of the pipette through the cervix and the possible inflammatory process triggered by it may stimulate premature prostaglandin (PGF₂α) release and a return of the animal to heat (TOBIN, 1979). Treatments based on the property of nonsteroidal anti-inflammatory drugs of inhibiting PGF₂α synthesis and altering the clinical function of the corpus luteum have been investigated (ARCHBALD, 1983; DARENIUS et al., 1989). Among these drugs, phenylbutazone (PBZ), extensively used by clinical veterinarians working with horses, inhibits the enzymes cyclooxygenase and hydroperoxidase, which are necessary for PGF₂α synthesis by arachidonic acid (MIZUNO et al., 1982). In adult horses, a 2 to 3 g intravenous dose of PBZ administered daily is sufficient to produce an anti-inflammatory effect and to inhibit PGF₂α synthesis (TOBIN, 1979). On the basis of these reported properties, we hypothesized that at the time of embryo transfer this drug may not only block the inflammatory reaction of the genital tract, but also the synthesis of PGF₂α, thus increasing the pregnancy rates of embryo recipient mares. These actions may also be maximized by maintaining adequate blood concentrations over a period of 24 hours by means of 3 doses applied at 8

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hour intervals. Thus, the objective of the present study was to investigate the effect of PBZ on the pregnancy rates of recipient mares presenting different degrees of uterine tonicity.

MATERIALS AND METHODS

The study was conducted on 32 Paint Horse breed donors and 96 Mangalarga recipients, belonging to three stud farms in the municipality of Uberaba, MG, during the 2001/2002 breeding season (October to February). Donor cycling mares, 4 to 8 years of age, were used. Once heat was detected, both donors and recipients were examined per rectum and monitored daily by ultrasound to determine the degree of follicular development and the exact day of ovulation. When a follicle measuring ≈ 35 mm in diameter was identified, the donors were inseminated every 48 hours using fresh semen with an inseminating dose of approximately 1.0×10^9 progressive spermatozoa.

Only recipients that ovulated one day before (-1) to three days after (+3) the donors were included in the study. The recipients were examined on day 5 post ovulation for the presence of a corpus luteum and the absence of endometrial edema or intrauterine fluid. Once approved, they were evaluated for uterine tonus by transrectal digital compression. Tonus was scored as T1 (absent), T2 (fair), T3 (moderate), and T4 (intense).

Embryos were collected on day 8 post ovulation of the donor using a silicone catheter (Veterinary Concepts, Spring Valley, WI) and uterine lavage with Ringer lactate solution (ALVARENGA et al., 1993). One liter of this solution was infused into the uterus by gravity and then drained into a collecting cup containing a 75 μ m filter (Veterinary Concepts, Spring Valley, WI). The procedure was repeated up to three times. After inspection and embryo location, the embryo was aspirated with a 1 ml syringe coupled to a sterile polyvinyl chloride 0.5 ml straw and

transferred to a Petri dish with a bottom divided into squares containing 12 small portions (4 drops each) of Emcare™ holding solution medium (ICP, Auckland, New Zealand). Embryos were then immediately washed as described by Imel et al. (1981).

Only embryos considered excellent or good were transferred using a horse insemination pipette coupled to a 3 mL syringe according to the technique of Iuliano et al. (1985). At the time of transfer, the recipients were divided into five groups and submitted to the following treatments:

Group A: Recipients with T1 and T2 uterine tonus (n = 19), which were not treated and received only the embryo.

Group B: Recipients with T1 and T2 uterine tonus (n = 14), treated with 1 dose of 2 g phenylbutazone^a iv. Group C: Recipients with T1 and T2 uterine tonus (n = 11) treated with 3 doses of 2 g of phenylbutazone iv at eight hour intervals.

Group D: Recipients with T3 and T4 uterine tonus (n = 23) treated with 1 dose of 2 g of phenylbutazone iv.

Group E: Recipients with T3 and T4 uterine tonus (n = 29), which were not treated and received only the embryo.

Pregnancy was diagnosed by rectal palpation and ultrasonography six days after transfer, i.e., at 14 days after the ovulation of the donor, and the examination was repeated at 20 and 35 days. The pregnancy rates obtained were analyzed statistically by the Chi-square test (χ^2 ; GOMES, 1985).

RESULTS

Pregnancy rates and numbers of recipient mares transferred per group are given in Table 1. The pregnancy rate for Group C did not differ significantly from Groups D and E but was superior to Groups A and B. However, at the first examination after embryo transfer, i.e., at 14 days post ovulation, the uterine tonicity was similar in all pregnant recipients (data not shown).

Table 1. Number of recipients transferred and pregnancy rates at day 35 for different groups

Groups of recipients	Doses of phenylbutazone (g)	Uterine tone ¹ transferred	Recipients (n)	Pregnancy rates (%)
A	0	T1 and T2	19	07/19 (36.8) ^a
B	1 x 2,0	T1 and T2	14	05/14 (35.7) ^a
C	3 x 2,0	T1 and T2	11	07/11 (63.6) ^b
D	1 x 2,0	T3 and T4	23	19/23 (82.6) ^b
E	0	T3 and T4	29	23/29 (79.3) ^b
Total			96	61/96 (63.5)

^{a,b} Values with different superscripts within columns differ significantly ($P < 0.05$).

¹ T1 = absent uterine tonus; T2 = fair uterine tonus; T3 = moderate uterine tonus; T4 = intense uterine tonus.

DISCUSSION

Recipients with absent or fair uterine tonus treated with 3 doses of PBZ at 8 hour intervals (Group C) showed a higher ($P < 0.05$) pregnancy rate compared to mares with the same tonus but treated with only 1 dose (Group B), demonstrating a possible effect of the increased number of doses and of the interval between them. This experimental design may also have been responsible for the absence of a significant difference in pregnancy rate between Group C and Groups D and E, since the mares in the last two groups had moderate to intense tonus. A single dose of PBZ applied to Group D did not cause a significant change in pregnancy rate, probably due to the presence of already adequate uterine tonicity. Although this is a speculative possibility, the increased pregnancy rate observed in Group C animals may be explained by the prolonged anti-prostaglandin action of PBZ obtained by the repetitions of the normal therapeutic dose. Thus protected against probable PGF₂ secretion induced by manipulation of the uterus (STABENFELDT et al., 1981), the newly transferred embryo would have more time to adapt to the uterine environment and to start the mobility phase for the maternal recognition of pregnancy. However, this hypothesis needs further investigation since Archbald et al. (1983), after treating mares with PBZ for 21 days, did not observe an effect on ovarian cyclicity or on plasma progesterone concentration.

Analysis of Table 1 shows that, regardless of treatment with PBZ, the animals with absent or fair uterine

tonus (Groups A, B and C) had lower pregnancy rates than animals with moderate to intense tonus (Groups D and E), confirming the results obtained by Carnevale et al. (2000). According to these investigators, the reduction of tonus may indicate a uterine environment not fully compatible with embryo development. Reduced uterine tonicity among recipients is related to low circulating progesterone levels (McCUE et al., 1999) and to unsatisfactory pregnancy rates (CARNEVALE et al., 2000). In the present study, we did not determine progesterone levels, a procedure that might have contributed to explain the pregnancy rates obtained.

On the other hand, the results of embryo transfer programs are affected by several factors, especially recipient variability, transfer method, embryo quality, and embryo manipulation. Considering the nonsurgical transfer method, the overall 63.5% pregnancy rate at 35 days (54/77) presented in Table 1 is considerably higher than that reported by Carnevale et al. (2000), i.e., 47.4% (37/78) and 39.7% (31/78) at 12 and 50 days, respectively. This was probably due to the fact that the cited investigators included in their study some mares considered to be old and subfertile, in the range age of more than 15 years, when higher embryo mortality is observed (WOODS et al., 1987).

The present results suggest a beneficial effect of PBZ applied at scheduled intervals to recipients with low uterine tonicity. However, due to the scarcity of information on the literature about the use of this drug for endocrine control of reproductive activities, further studies are needed in order to determine its exact mechanism of action and implications.

RESUMO: O objetivo desse experimento foi avaliar as taxas de prenhez de receptoras com graus variados de tonicidade uterina (ausente, fraco, moderado e intenso) tratadas com fenilbutazona (FBZ). Foram utilizadas 32 éguas doadoras da raça Paint Horse e 96 receptoras Mangalarga no ano hípico 2001/2002 (outubro a fevereiro). Os embriões foram colhidos no 8º dia pós-ovulação, classificados e transferidos em seguida pelo método não cirúrgico. No momento da transferência as receptoras foram separadas em grupos de A a E, em função da tonicidade uterina. O Grupo A (n = 19) consistiu de receptoras com tônus ausente ou fraco, que não foram tratadas e receberam apenas o embrião; o Grupo B (n = 14) consistiu de receptoras com tônus ausente ou fraco, tratadas com 1 dose de 2 g de FBZ; o Grupo C (n = 11) consistiu de receptoras com tônus ausente ou fraco, tratadas com 3 doses de 2 g de FBZ, a cada oito horas; o Grupo D (n = 23) consistiu de receptoras com tônus moderado ou intenso, tratadas com 1 dose de 2 g de FBZ; o Grupo E (n = 29) consistiu de receptoras com tônus moderado ou intenso, que não foram tratadas e receberam apenas o embrião. Aos 35 dias após a transferência do embrião a taxa total de prenhez foi de 63,5%, sendo de 36,8% (07/19); 35,7% (05/14); 63,6% (07/11), 82,6% (19/23) e 79,3% (23/29) para os grupos A, B, C, D e E, respectivamente. A taxa de prenhez do grupo C não diferiu significativamente dos Grupos D e E, mas foi superior ($P < 0,05$) aos Grupos A e B, evidenciando que a FBZ, em três aplicações repetidas a cada 8 horas, pode ser benéfica às taxas de prenhez de receptoras com baixa tonicidade uterina.

UNITERMOS: Fenilbutazona, Transferência de Embriões, Receptora, Éguas.

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