Effect of feeding fermented rice punch on oestrous induction in anoestrous bitches

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ABSTRACT

Introduction: As a consequence of poor productivity caused by a long anoestrous period, considerable research effort has been given to oestrous induction in dogs to enhance the productivity of young dogs and to preserve breeds.

Materials and methods: Oestrous was induced in 30 anoestrous bitches more than three months after the last oestrus. Bitches orally received fermented rice punch with or without bromocriptine once daily for 21 consecutive days. The bitches were divided into two groups (n=10 per group): Group (1) fed fermented rice punch and Group (2) administered bromocriptine (100 µg/kg/day) and fed fermented rice punch.

Results: The concentration of dopamine in fermented rice punch was 47.2 mg/kg (parts per million). Six of 10 (60.0 per cent) and seven of 10 (70.0 per cent) bitches showed pro-oestrous bleeding in Groups 1 and 2, respectively. The mean and median values (min–max) to oestrous induction was not significantly different between Groups 1 and 2 (9.7±7.3, 6.5 (3–22) and 11.3 ±6.6, 7.9 (5–21) days) after treatment commencement (P>0.05). The pregnancy rate was very similar between Groups 1, 2 (66.0%) and control (66.0, 57.0 and 50.0 per cent). The mean and median values (min–max) of pups per bitch are also not significantly different between Groups 1, 2 and control (7.5±2.1, 7.5 (5–10) and 7.0±0.7, 7.0 (7–10)).

Conclusion: We suggest that rice punch effectively induces oestrus in bitches.

INTRODUCTION

The oestrous cycle of canidae differs considerably from that of other species. Domestic bitches are non-seasonally monoestrus and, as a result of this unique reproductive physiology, exhibit cyclicity only once or twice per year. In domestic bitches, the follicular phase and spontaneous ovulation are followed by a luteal phase with an average duration of about 75 days (Schaefers-Okkens 1996). A non-seasonal anoestrous of a variable duration (2–10 months) follows each oestrous cycle (Bouchard and others 1991, Concannon 1995). There are three categories of drug treatment for oestrous induction in dogs. The first category involves stimulating follicular development and includes the use of pregnant mare serum gonadotropin (PMSG), follicle-stimulating hormone (FSH), luteinizing hormone (LH) and human menopausal gonadotropin. The most widely studied gonadotropin for oestrous induction in bitches is PMSG, with protocols ranging from daily to weekly subcutaneous or intramuscular injections (Concannon 1993, Cirit and others 2007a, b, Kutzler 2007, Weilenmann and others 1993). The second category involves stimulating the release of pituitary gonadotropins (gonadotropin-releasing hormone and its analogues) and oestrogen. The third category involves shortening anoestrous periods by inhibiting the synthesis or release of prolactin using dopamine agonists (Arnold and others 1989, Bouchard and others 1991, Cirit and others 2007b, Johnston and others 2001). Prolactin influences the length of the canine inter-oestrous interval via its effects on gonadotropin secretion and the responsiveness of ovaries to gonadotropins.

Dopamine agonists can shorten the duration of anoestrus or induce oestrus in dogs with a prolonged anoestrous period (Gobello and others 2002, Feldman and Nelson 2004, Cirit and others 2007b). However, cabergoline and bromocriptine, the commonly used dopamine agonists, cause various side effects such as vomiting and nausea. Vomiting is a frequently observed side effect (3–25 per cent of cases) within one hour after the first treatment with bromocriptine or cabergoline (Arbeiter and others 1988, Jochle and others 1989, Verstegen and others 1999). In rats, dopamine agonists and antagonists affect ovarian steroidogenesis (Mori and others 1994). Inspired by this point in the mouse germ (Malt) fed a control object, and the concentration of prolactin in mice decreases after they giving birth (Song and others 1994). During oestrous synchronisation upon the administration of a dopamine agonist or dopamine, blood FSH
concentration increases as the result of reduced prolactin concentration in the blood (Concannon 1998). Bitches are multioestrous animals; however, the mechanisms controlling their oestrous cycle are unclear and there is no means to stimulate multiple concurrent oestrous periods (Ajikumar and others 2010). The purpose of inducing oestrus in bitches is to conserve the genetic traits of pedigree dogs. Fermented rice punch plays a pivotal role in reducing the secretion of milk by cows and mice (Song and Lee 1994). During the initial stage of oestrus, blood prolactin concentration and milk secretion are reduced and blood FSH concentration is increased (Arthur and others 1996). Therefore, the objective of this study was to evaluate whether artificial oestrus can be induced by feeding anoestrous bitches fermented rice punch and to determine the effect of this supplementation on pregnancy rates and litter size.

MATERIALS AND METHODS
Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Animals were cared for and used in accordance with the Gyeongsang National University Guidelines for the use of laboratory animals (approval no. GNU-131105-D0066).

Twenty four pit bull terrier bitches (age, 1.5–2.5 years; weight, 25–38 kg (mean 31.5 kg)) were eligible for inclusion in this study. The blood progesterone concentration was determined in all bitches; those with a concentration <2 ng/ml were considered to be anoestrous. The study also included bitches that gave birth a minimum of 90 days before commencement of the treatment and had not become pregnant since then.

A total of 20 anoestrous bitches were divided by random selection into two groups: Group (1) fed with rice punch (1 l/day) orally for 21 days and Group (2) fed with rice punch (1 l/day) and was administered bromocriptine (100 µg/kg/day) orally for 21 days, and additional four pro-oestrous bleeding bitches were included as control. Rice punch was produced by adding 35 kg of malt powder to 200 l of water. Thereafter, 4 kg of sugar was added per 10 kg of rice and the mixture was incubated for more than six hours at 55°C. The rice punch was stored at −20°C and defrosted on the day of use.

High-performance liquid chromatography (HPLC) was used to examine dopamine agonists (cabergoline, bromocriptine and pergolide) and dopamine in fermented rice punch. Samples were subjected to the methanol ultrasonic extraction method using a 0.45 µm polytetrafluoroethylene filter. HPLC was performed using a Waters 1525 Binary HPLC Pump, a Waters 2707 Automatic Sample Injection System and a ZORBAX Eclipse XDB C18 Waters 2489 UV detector. HPLC column (4.6×250 mm). The solvents used were water containing 1% formic acid and an acetonitrile: methanol:water mixture (4.5:4.5:1, v/v). The flow rate was 1.0 ml/minute and the sensing device was a UV detector (250, 270 nm). The injection volume was 10 µl and data processing was performed using the Breeze 2 program.

Blood samples were collected in 5 ml collection tubes on days 1, 3, 5 and 7 of oestrus from at least three dogs in two treated groups and natural oestrous group as control. The samples were kept at room temperature for a few minutes and then centrifuged for five minutes at 2570 g. The separated serum was stored at −20°C. The serum was thawed slowly to measure the concentrations of FSH and progesterone using a miniVIDAS instrument (BIOMERIEUX, Paris, France).

During the experimental period, treatment (rice punch with or without bromocriptine) was continued until oestrus was induced, up to a maximum of 21 days. The cycle stage and timing of the fertile period were determined by several observable parameters (vulvar swelling, discharge, behavioural changes and receptivity) and vaginal cytology (Papanicolaou staining of vaginal smears). We then conducted artificial insemination to all of the bitches that were in oestrus after induction (bitches from Groups 1 and 2) or natural oestrus (control).

Dog semen was collected from male dogs using an artificial vagina. After collection of semen, seminal fluid was removed using a medical syringe without a needle. A 1.4 inch vaginal tube was inserted into the vagina of the bitch as previous report (Sánchez and others 2011). The syringe containing semen was attached to the outer part of the tube. The plunger was slowly pushed down as the tube was held upright until all the semen was delivered into the vagina. The number of live pups was counted after 60–63 days of pregnancy and natural delivery.

All data were analysed using the Statistical Package for the Social Sciences software package for Windows (SPSS V.18; SPSS, Chicago, Illinois, USA). The treatment groups were analysed by an analysis of variance (ANOVA) using the general linear model procedure. Results are expressed as the percentage, mean±SD and median number. The number of pro-oestrous bleeding and pregnancy, oestrous induction time and number of live pups between groups were analysed by a one-way ANOVA followed by multiple pairwise comparisons followed by Duncan’s multiple range test. The Box and Whisker Plots were created using Microsoft Excel 2010. P<0.05 was considered significant.

RESULTS
A total of 20 anoestrous bitches that were more than three months after the last oestrus were considered to be in anoestrus and were included for oestrus induction and an additional four dogs were included as control. We found that six of 10 (60 per cent) and seven of 10 (70 per cent) bitches showed pro-oestrous bleeding in Groups 1 and 2, respectively (Table 1). The mean and median values of days (min–max) after treatment commencement needed 9.7±7.3, 6.5 (3–22) and 11.3±6.6, 7.0 (5–21) in Groups 1 and 2, respectively. The time to oestrous induction was not significantly different between Groups 1 and 2 (P>0.05).
The concentration of dopamine in fermented rice punch was 47.2 mg/kg (parts per million, Fig 1). The plasma concentration of progesterone at oestrus days 3, 5 and 7 was significantly higher than those at start and day 1, and also significantly increased according to days 3, 5 and 7 (P<0.05, Fig 2). The plasma concentration of FSH at oestrus days 1, 3, 5 and 7 was significantly higher than that at start day (P<0.05), but did not differ among days 1, 3, 5 and 7. However, FSH concentration of group 1 at day 7 was significantly lower than control and group 2 (P<0.05, Fig 3).

We found that pregnancy rate was not significantly different between Groups 1, 2 and control (66, 57 and 50 per cent respectively, P>0.05). The mean and median values of pups number (min–max) per bitch was very similar between the groups (7.0±1.8, 7.0 (5–9) and 7.5±2.1, 7.5 (5–10) and 7.0±0, 7.0 (7–7)) in Groups 1, 2, and control, respectively; P>0.05; Table 1).

**DISCUSSION**

The aim of this study was to assess the ability of fermented rice punch, with or without administration of bromocriptine, to induce oestrus in anoestrous bitches. The mean and median values of days from treatment commencement to onset of pro-oestrus was not different between the fermented rice punch group (9.7±7.3, 6.5 (3–22) days) and bromocriptine (100 μg/...

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**TABLE 1:** Comparison of the efficiency of oestrous induction and pup production among the three groups

| Treatment*                           | Group 1       | Group 2       | Control |
|--------------------------------------|---------------|---------------|---------|
| Number of bitches that showed pro-oestrous bleeding (%) | 6 (60.0)a     | 7 (70.0)a     | 4       |
| Mean and median values of days between treatment commencement and oestrous induction (min–max) | 9.7±7.3, 6.5 (3–22)a | 11.3±6.6, 7.0 (5–21)a |
| Number of bitches that became pregnant after artificial insemination (%) | 4 (66.0)a     | 4 (57.0)a     | 2 (50.0)a |
| Mean and median values of live pups born per bitch (min–max) | 7.0±1.8, 7.0 (5–9)a | 7.5±2.1, 7.5 (5–10)a | 7.0±0, 7.0 (7–7)a |

Values with same superscript a in same row were not significantly different (P<0.05).

*Group 1 and 2 contained 10 dogs.

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**Fig 1** High-performance liquid chromatography analysis of fermented rice punch.
kg body weight/day) plus fermented rice punch (11/day) group (11.3±6.6, 7.0 (5–21) days). The percentage of bitches that showed pro-oestrual bleeding was not significantly different between bitches that received both bromocriptine and fermented rice punch (70%, 7/10) and bitches that received fermented rice punch alone (60%, 6/10). Such results indicate that feeding anoestrous bitches with fermented rice punch is effective in induction of oestrus. This result is consistent with previous studies, which demonstrated that bromocriptine treatment induces oestrus in 80–100 per cent of bitches (van Haaften and others 1989; Zöldag and others 2001). Verstegen and others (1999) reported that 93.33 per cent of bitches exhibited pro-oestrus responses following treatment with cabergoline.

As mentioned previously, dopamine induces oestrus by lowering the blood prolactin concentration. This indicates that dopamine in fermented rice punch affected the induction of oestrus. This result is in agreement with the finding that blood prolactin concentration was lower in mice fed fermented rice punch than in untreated mice (Song and Lee 1994). Dopamine analogues were detected in the fermented rice punch used in this study. Fermented rice punch induces oestrus via its anti-prolactin actions. Based on these results, we propose that dopamine and dopamine analogues in fermented rice punch induce oestrus in anoestrus bitches. Dopamine agonists induce fertile oestrus in bitches of most breeds. Van Haaften and others (1989) reported that proestrus onset in bitches occurred after 28 days of bromocriptine treatment. Other studies demonstrated that oestrus was induced in bitches after 29.75±5 days (Jeukenne and Verstegen 1997) and 16 days (Gobello and others 2002) of treatment with cabergoline. Furthermore, in the current study, it was confirmed that the time between treatment commencement and oestrous induction was shorter in bitches administered rice punch alone than in untreated bitches.

Progesterone concentration begins to rise at approximately the time of the LH surge (before ovulation). The increasing progesterone concentration acts synergistically with the declining oestrogen concentration to reduce oedema of the vulva and vagina (Arthur and others 1996). The progesterone concentration typically increases by >2 ng/ml every two days during the LH surge of oestrus (Grundy and others 2002). In the current study, the progesterone concentration increased.
steadily in all groups (Fig 2), consistent with the other findings, but did not show differences on the same day of each group. The similar progesterone concentration among groups in the same day is normal oestrus induction by feeding of fermented rice punch. The oestrogen level is of little value for ovulation timing because the peak oestrogen level varies among bitches. The oestrogen level does not indicate the fertile period of a bitch because ovulation is triggered by the LH surge, not by the oestrogen peak (Grundy and others 2002).

Mating or artificial insemination of bitches (bromocriptine treatment) resulted in an 83% pregnancy rate (40 cases) and 39 (97.5%) of them gave birth to puppies (Zöldag and others 2001). In this study, the pregnancy rate was not different between Groups 1, 2 and control. The mean number of pups per bitch was not significantly different between the Groups 1, 2 and control (7.0, 7.5 and 7.0, respectively; P>0.05). In some studies, bromocriptine alone was used; the average litter size was small with 4.8±1.6 pups (Zöldag and others 2001), and in some others, carbergoline alone was used; the average litter size was 5.8±1.94 (Cirit and others 2007b) and 6.6±0.2 (Nak and others 2012) and was very similar with data in the current study. The litter size in oestrous induction groups was very similar with natural oestrous group. This suggests that the induction of oestrous level results in similar litter size when compared with naturally occurring oestrus.

In conclusion, we demonstrated that rice punch effectively induces oestrus in bitches. Furthermore, this treatment regimen has no side effects, such as causing nausea and vomiting, and contains natural oestrus-inducing ingredients.

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Contributors I-KK participated in the design of the study, data acquisition, performed data analysis and drafted the manuscript. P-RP participated in performing the study. Both authors read and approved the final manuscript.

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