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The effect of oxytocin and prostaglandin hormones added to semen on stallion sperm quality

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Abstract: Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and oxytocin have been used to improve reproductive performance in many mammalian species, including humans. The aim of the present study was to determine their effects on sperm quality when extended stallion semen was enriched with $PGF_{2\alpha}$ or oxytocin. In this study, 16 healthy adult stallions, each 15–22 years old, were used. Five ejaculates from each stallion were collected with an artificial vagina during breeding season. Thereafter, gel-free semen was divided into 7 aliquots and diluted with an INRA96 semen extender. The following different treatments were evaluated: 3 with only $PGF_{2\alpha}$ (10, 20, and 40 µg/mL) and 3 with only oxytocin (2, 3, and 4 IU/mL). Sperm quality was evaluated before and after applying the different treatments. In vitro addition of oxytocin to semen did not show improvement in any of the sperm quality parameters measured. However, sperm supplementation with 40 µg of $PGF_{2\alpha}$ caused a significant increase ($P < 0.05$) in diluted semen motility. We concluded that the addition of $PGF_{2\alpha}$ to elder stallions' semen may help maintain sperm motility. Future research might assess the effects of $PGF_{2\alpha}$ on fertility. Our results were based on in vitro evaluations, and thus further fertility trials are required.

Key words: Oxytocin, prostaglandin, semen, stallion

1. Introduction

The number of mares in artificial insemination or natural breeding depends on the quality and quantity of stallion semen. Stallions will typically remain fertile beyond their 20th year of age. When using elder stallions in artificial insemination or natural breeding, the quality and/or quantity of semen tend to decline (1). The fertility of a stallion depends on age, testicular volume, and the sperm reserve capacity of the epididymis; it is also influenced by extrinsic factors such as nutrition, temperature, breeding season, frequency of ejaculation, and medication (2). Stallion fertility is of high economic importance for the horse industry and infertility results in huge economic losses to the breeder (3). Numerous pharmacological agents like $PGF_{2\alpha}$, oxytocin, and gonadotropin releasing hormones have been shown to enhance male reproductive performance in many species (4,5).

The function of prostaglandins found in seminal plasma has not been fully documented (6), but some researchers have reported that the prostaglandins present in semen may play a role in uterine clearance and/or sperm transport (7,8). It was found that the supplementation of semen with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) increased the rate of sperm motility in humans and boars, while some researchers reported that the addition of $PGF_{2\alpha}$ to diluted boar semen or human semen did not affect sperm motility (9).

Oxytocin was detected in all fractions of the stallion semen and was highest in the gel. The exact function of oxytocin in the male animal is not clear, but it has been suggested that oxytocin is involved in sperm transport and motility in domestic animals (10). Previous studies have shown that the effect of $PGF_{2\alpha}$ and oxytocin treatment on sperm quality in many mammalian species, including humans, is contradictory (8,11–13). The aim of the present study was to determine the effects of in vitro addition of oxytocin (Oksitosin, Vetaş) or $PGF_{2\alpha}$ (Dinolytic, Pfizer) on semen quality in 16 stallions, aged between 15 and 22 years.

2. Materials and methods

2.1. Animals and experimental design

The study was performed using 16 healthy warmblood stallions, aged between 15 and 20 years, from the Karacabey Stud Farm in Bursa, Turkey. The animals were kept in box stalls bedded with straw and were fed hay, oats, and pellets

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supplemented with minerals. Water was freely available. All animals were exercised daily for at least 1 h. Five ejaculates from 16 warmblood healthy stallions were used to analyze the effects of $PGF_{2\alpha}$ and oxytocin on stallion sperm quality. The study was approved by the Ethical Committee of Ankara University Veterinary Faculty. Semen samples were collected from the stallions during the breeding season from March to June. In the first week, semen samples were collected using a Missouri model artificial vagina on an estrous mare from each stallion to stabilize extragonadal sperm reserves. Spermatological parameters (semen volume, sperm concentration, total sperm motility, abnormal sperm, and membrane integrity) were recorded as control values.

2.2. $PGF_{2\alpha}$ and oxytocin treatments

In the second week, each stallion semen sample (n 16) was divided into 7 equal parts and extended to a concentration of 50×10^6 spermatozoa/mL in an INRA96 semen extender either supplemented with oxytocin (2, 3, and 4 IU/mL) or prostaglandin (10, 20, and 40 µg/mL), or containing no oxytocin and prostaglandin. The samples were mixed thoroughly and the sperm quality was evaluated 5 min after the addition of $PGF_{2\alpha}$ or oxytocin.

2.3. Semen processing and examination

After semen samples were collected using a Missouri model artificial vagina, the gel fraction was filtered (US BAG; Minitube of America, Inc.) to remove the gel. The gel-free volume and spermatological parameters were determined. Sperm motility was assessed using a phase-contrast microscope (400× magnification) (Olympus BH-2, Olympus Optical Co. Ltd.), with a warm stage maintained at 37 °C. A wet semen mount was made using 5 µL of semen placed directly on a microscope slide and covered by a cover slip. For each sample, at least 5 microscopic fields were examined by 2 trained observers. The mean of the 3 successive evaluations was recorded as the final motility score. The concentration of spermatozoa was determined using the hemocytometric method, after diluting the semen with Hayem's solution (dilution rate: 1/500). Sperm morphology assessment was identified by Giemsa staining. One drop of ejaculate or 5–10 µL was placed on the slide and a thin smear was made using the edge of another glass slide or cover slip. The smear was air-dried. The slide was dipped in the Giemsa stain for 3–5 min and washed under running tap water and then dried in air. The percentage of sperm abnormalities was recorded under immersion oil at 100× microscope magnification by counting 200 sperm cells with bright-field microscopy (400×) (Olympus BH-2, Olympus Optical Co. Ltd.; oil immersion). The hypoosmotic swelling test was used to evaluate the functional integrity of the sperm membrane. The test was performed by incubating 10 µL of semen in 100 µL of semen of a 100 mOsm hypoosmotic solution (fructose and sodium citrate) at 37 °C for 30 min. Following incubation, 0.1 mL of the mixture was spread with a cover slip on a warm slide. The sperm was evaluated using bright-field microscopy (Olympus C X 21FS1, Olympus Optical Co. Ltd) and all sperm cells with swollen or coiled tails were recorded.

2.4. Statistical analyses

Data from each ejaculate from each stallion (every ejaculate was considered as a case; n = 16) were managed using Microsoft Excel and SPSS for Windows, version 14.1. These variables were assessed in the control samples and compared with the treatment conditions using either one-way ANOVA or a Shapiro–Wilk test when, even transformed, the distribution of the variables was abnormal. The significance was set at $P < 0.05$.

3. Results

The spermatological characteristics of nondiluted fresh semen and diluted semen containing different amounts of $PGF_{2\alpha}$ or oxytocin are presented in the Table. The addition of different doses of oxytocin to the semen extender did not alter the semen parameters. Sperm supplementation with 40 µg of $PGF_{2\alpha}$ caused a significant increase ($P < 0.05$) in the motility of diluted semen. The addition of 10 or 20 µg of $PGF_{2\alpha}$ did not show significant ($P > 0.05$) improvement in sperm motility. There were no significant differences between the treatment groups in the integrity of the sperm membrane and sperm morphology.

4. Discussion

The goal of this study was to evaluate the effects of $PGF_{2\alpha}$ and oxytocin on stallion sperm quality. Many investigations have been carried out to determine the effects of hormones like prostaglandin and oxytocin on sperm function (9,14,15). In the present study, the addition of different amounts of oxytocin to semen did not alter the sperm quality. Clough et al. showed that 5 min after the addition of 30 IU of oxytocin to an extender for stallion semen there was no deleterious effect, but it did not improve sperm motility either (11). In another experiment, the addition of 2.5 IU, 5.0 IU, and 7.5 IU/100 mL oxytocin to Niliravi buffalo ejaculates did not improve semen quality after 24 h of deep freezing (12). The present results are consistent with the findings reported by these authors. Our results are unlike previous work on the use of oxytocin in semen extenders, which was related to the possibility of improving sperm motility rather than promoting uterine contractions (11). Moreover, the addition of oxytocin in extended semen just before artificial insemination was shown to improve fertility (16) and the farrowing rate of sows (17,18).

In the present study, we observed that the addition of 40 µg/mL $PGF_{2\alpha}$ to diluted semen caused a significant

Table. Mean (±SE) sperm quality from 16 stallions in extenders with and without oxytocin or $PGF_{2\alpha}$ supplementation (n = 16).

Treatment (n = 16)	Motility (%)	Total abnormality (%)	Membrane integrity (%)
2 IU/mL oxytocin	55.20 ± 9.07[a]	63.31 ± 4.37[a]	65.41 ± 4.98[a]
3 IU/mL oxytocin	50.32 ± 4.38[a]	65.97 ± 1.23[a]	64.78 ± 3.39[a]
4 IU/mL oxytocin	49.73 ± 558[a]	62.32 ± 4.92[a]	63.67 ± 3.76[a]
10 µg/mL $PGF_{2\alpha}$	55.42 ± 5.51[a]	60.20 ± 3.68[a]	65.26 ± 4.05[a]
20 µg/mL $PGF_{2\alpha}$	55.10 ± 7.44[a]	63.41 ± 4.43[a]	67.41 ± 4.77[a]
40 µg/mL $PGF_{2\alpha}$	65.70 ± 8.18[b]	65.43 ± 4.09 [a]	63.55 ± 3.76[a]
Control	55.5 ± 7.25[a]	66.00 ± 1.50[a]	67.89 ± 5.54[a]

Groups with different letters (a, b) in the same column are significantly different ($P < 0.05$).

increase in sperm motility, while the addition of 10 or 20 µg/mL $PGF_{2\alpha}$ to semen did not affect sperm motility. Contradictory findings have been reported in a series of studies previously (9,19). The addition of $PGF_{2\alpha}$ (0, 33, 63, 94, or 125 µg/mL) in semen extenders during incubation for 15 and 240 min at 37 °C and then for 24 h at 5 °C for stallions was not found to alter sperm motility and showed no beneficial effect on fertility (19). Conversely, Karahan reported that the supplementation with 125 or 250 µg/mL $PGF_{2\alpha}$ caused a significant increase in the motility of diluted bull semen stored at 4 °C after 24 h as compared with diluted $PGF_{2\alpha}$-free semen (9). Additionally, Pandur and Pacala reported that sperm motility, after the addition of $PGF_{2\alpha}$ (Dinolytic; 5 mg/mL $PGF_{2\alpha}$) to landrace boar diluted semen, was enhanced. (20). The effect induced by the addition of $PGF_{2\alpha}$ in the present study was consistent with the findings reported by other investigators. These increases in sperm motility can be explained by the direct effect of prostaglandins on spermatozoa, possibly acting on the contractile elements of the sperm and stimulating its kinetic activity. However, some researchers alleged that the addition of high amounts of $PGF_{2\alpha}$ to semen had detrimental effect on sperm motility (9,13,21). Fayed reported that the supplementation of diluted bull spermatozoa with high levels of $PGF_{2\alpha}$ (300, 600, or 1200 µg/mL) suppressed sperm motility and induced sperm membrane damage and permeability (13). These decreases in sperm motility may be explained by stimulation of the production of cyclic guanosine monophosphate (9).

In summary, the addition of oxytocin did not show any positive or negative effects on stallion semen parameters. The inclusion of $PGF_{2\alpha}$ in semen extenders for older stallions was found to affect sperm motility. Therefore, it is suggested that $PGF_{2\alpha}$ could be added to semen in small amounts as it might improve sperm motility in stallions aged between 15 and 22 years at the time of insemination. Further research should be conducted to determine whether exogenous $PGF_{2\alpha}$ could be administered to stallions at the time of insemination or added to the extended semen to enhance conception rates.

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