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Research Article

In Vitro Effects of Prostaglandin $F_{2\alpha}$ and Metamizol on the Motility of Diluted Bull Semen

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Abstract: The purpose of the present study was to investigate in vitro effects of prostaglandin F_2 alpha ($PGF_{2\alpha}$) and metamizol on the motility of diluted bull semen during short-term storage at 4 ºC. In this study, 3 healthy adult Brown-Swiss bulls, each 3 years old, were used. Semen samples were collected with an artificial vagina. Samples were taken from each bull twice a week over the course of 3 weeks (6 samples per bull). One millilitre of semen was removed from each sample for the determination of semen volume, mass activity, initial motility, sperm concentration, and abnormal sperm rate. The remainder of the semen samples of each bull were diluted to a concentration of 1:10 (semen:extender) with isotonic sodium citrate + egg yolk solution. The diluted semen samples were then divided into 12 sterile tubes of equal volume (0.5 ml). Different amounts of $PGF_{2\alpha}$ [0 ($PGF_{2\alpha}$-free), 125, 250, 500, 1000, and 1500 µg] were added to 6 of these samples at 37 ºC. Similarly, different amounts of metamizol [0 (metamizol-free), 125, 250, 500, 1000, and 1500 µg] were added to the other 6 samples at 37 ºC. After the supplementation of $PGF_{2\alpha}$ or metamizol, all semen samples were stored at 4 ºC. The sperm motility of the samples was examined at 0, 1, 2, 4, 24, 48, 72, 96, 120, 144, 168, 192, and 216 h.

No significant differences in spermatological characteristics were determined between the bulls. Sperm supplementation with 125 µg and 250 µg $PGF_{2\alpha}$ caused a statistically significant increase ($P < 0.05$) in the motility of diluted semen stored at 4 ºC after 24 h versus diluted $PGF_{2\alpha}$-free semen. However, the supplementation with 500 µg and 1000 µg $PGF_{2\alpha}$ caused significant motility decreases after 4 h, while 1500 µg $PGF_{2\alpha}$ also caused a decrease after 2 h compared to diluted $PGF_{2\alpha}$-free semen ($P < 0.05$). On the other hand, metamizol supplementation of 125 µg and 250 µg significantly decreased the motility of diluted semen stored at 4 ºC after 48 hand 24 h, respectively, while 500 µg, 1000 µg, and 1500 µg of metamizol reduced motility after 4 h ($P < 0.05$) in comparison to the diluted metamizol-free semen.

In conclusion, the addition of small amounts of $PGF_{2\alpha}$ to diluted bull semen samples may help to maintain sperm motility during short-term storage at 4 ºC.

Key Words: Bull, semen, $PGF_{2\alpha}$, metamizol, sperm motility

Sulandırılmış Boğa Spermalarının Motilitesi Üzerine Prostaglandin $F_{2\alpha}$ ve Metamizol'un İn Vitro Etkileri

Özet: Bu çalışma, 4 ºC'da kısa süreli saklanan sulandırılmış boğa spermasının motilitesi üzerine prostaglandin F_2 alfa ($PGF_{2\alpha}$) ve metamizolun in vitro etkilerini araştırmak amacıyla yapıldı. Araştırmada, 3 yaşında, sağlıklı 3 adet İsviçre Esmeri boğa kullanıldı. Her bir boğadan 3 hafta boyunca haftada iki kez olmak üzere 6 sperma örneği alındı. Her sperma örneğinin 1 ml'si sperma miktarı, kitle hareketi, başlangıç motilitesi, spermatozoon yoğunluğu ve anormal spermatozoon oranı'nın belirlenmesi için ayrıldı. Her boğanın sperma örneklerinin geri kalanı 1:10 oranında (sperma:sulandırıcı) izotonik sodyum sitrat+yumurta sarısı sulandırıcısıyla sulandırıldı. Daha sonra sulandırılmış sperma örnekleri eşit hacimde (0,5 ml) 12 steril tüpe konuldu. Bu örneklerin 6 tanesine $PGF_{2\alpha}$'nın farklı miktarları (0, 125, 250, 500, 1000 and 1500 µg), benzer şekilde metamizol de aynı miktarlarda diğer 6 örneğe 37 ºC'de ilave edildi. $PGF_{2\alpha}$ ve metamizol ilavesinden sonra sperma örneklerinin tümü 4 ºC'de saklandı. Bu örneklerde spermatozoon motilitesi 0, 1, 2, 4, 24, 48, 72, 96, 120, 144, 168, 192 ve 216. saatlerde belirlendi.

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Spermatolojik özellikler yönünden boğalar arasında başlangıçta bir farklılık olmadığı tespit edildi. Sulandırılmış ve 4 ºC'de saklanan sperma örneklerine 125 ve 250 µg $PGF_{2\alpha}$'nın ilave edilmesi, $PGF_{2\alpha}$ bulunmayan sulandırılmış sperma örneklerine göre 24. saatten sonra spermatozoon motilitesinde istatiksel olarak anlamlı ($P < 0,05$) bir artış oluşturdu. Bununla birlikte, $PGF_{2\alpha}$ bulunmayan sulandırılmış sperma örneklerine göre, 500 ve 1000 µg $PGF_{2\alpha}$ ilave edilmesi 4. saatten sonra, 1500 µg $PGF_{2\alpha}$ ilave edilmesi ise 2. saatten sonra spermatozoon motilitesinde anlamlı ($P < 0,05$) azalmalara neden oldu. Diğer yandan, metamizol bulunmayan sulandırılmış sperma örnekleriyle karşılaştırıldığında 500 ve 1000 µg metamizol ilave edilmesi sırasıyla 48 ve 24. saatten sonra; metamizol'un 500, 1000 ve 1500 µg'lık miktarları ise 4. saatten sonra 4 ºC'de saklanan sperma örneklerinin motilitesinde anlamlı ($P < 0,05$) azalmalara neden oldu.

Sonuç olarak, sulandırılmış boğa sperma örneklerine $PGF_{2\alpha}$'nın düşük miktarlarda ilave edilmesinin 4 ºC'de kısa süreli saklanması esnasında motilitenin daha uzun süre korunmasına yardımcı olabileceği kanaatine varıldı.

Anahtar Sözcükler: Boğa, sperma, $PGF_{2\alpha}$, metamizol, spermatozoon motilitesi

Introduction

Prostaglandins (PGs), which consist of 20-carbon unsaturated fatty acids, have been found in many mammalian tissues. PGs have a wide variety of actions, including $PGF_{2\alpha}$, which has an important role in female and male reproductive systems (1,2). In females, $PGF_{2\alpha}$ is formed in the granulose cells of the preovulatory follicle in response to gonadotropins, is synthesised in the oviduct, and contracts uterine muscle in vivo and in vitro. The role of $PGF_{2\alpha}$ in the male reproductive system, especially in semen, is still conjectural (3,4); however, seminal plasma is the major source of PGs (5), which are synthesised in the seminal vesicles and secreted into seminal plasma (6) and the prostate, whereas the testes synthesise only small amounts (7).

The presence of high concentrations of PGF in human seminal fluid (6,8) or the addition of high amounts of $PGF_{2\alpha}$ to the semen of humans (9) and bulls (10) is associated with poor sperm motility. It was found that the supplementation of semen with $PGF_{2\alpha}$ increases the rate of sperm motility in humans (11) and boars (12), whereas some researchers reported that human semen $PGF_{2\alpha}$ (13,14) or the addition of $PGF_{2\alpha}$ to diluted boar semen (15) does not affect sperm motility. It has been documented that while intramuscular injection of $PGF_{2\alpha}$ does not affect sperm motility in dogs (16) and horses (17), this application decreases sperm motility in rams (18). On the other hand, PGs cause suppression of spermatogenesis, either by acting directly on the seminiferous tubules or through interference with the secretion of testosterone (19).

Metamizol has analgesic, antipyretic, and anti-inflammatory effects. It is used mainly for the relief of minor pain, particularly of musculoskeletal origin (20). Some studies indicate that drugs interfering with PG metabolism, such as non-steroidal anti-inflammatory substances, may affect male reproductive functions (21,22). Metamizol and other anti-inflammatory substances, such as acetylsalicylic acid and indomethacin, are potent inhibitors of cyclooxygenase, which mediates prostaglandin biosynthesis. Inhibition of cyclooxygenase prevents biosynthesis of PGs (23), and may affect sperm motility (21,24). It has been reported that the administration of metamizol, indomethacin, and acetylsalicylic acid affects sperm motility of several species (18,24,25).

As mentioned above, the data regarding the in vivo or in vitro effect of $PGF_{2\alpha}$ on sperm motility are contradictory, and there are no data about the effect of metamizol on the motility of bull semen, in vivo or in vitro. Therefore, the present study was conducted to investigate in vitro effects of $PGF_{2\alpha}$ and metamizol on the motility of diluted bull semen during short-term storage.

Materials and Methods

Chemicals

$PGF_{2\alpha}$ (Etiproston, Prostavet-C, 5 mg / 2 ml) was supplied by Virbac (Carros, France) and metamizol (Metamizol sodium, Novalgin, 1 g / 2 ml) was obtained from Aventis (İstanbul, Turkey). All other chemicals were purchased from Sigma (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Animals

Three healthy adult Brown-Swiss bulls, each 3 years old, were used in the study. The bulls, were raised at the

Centre of Education, Research, and Application at the Faculty of Veterinary Medicine, Fırat University, and were kept under the natural climate conditions in Elazığ province, Turkey (lat 38°40'N). The bulls were fed concentrated meal supplemented with lucerne hay and fresh drinking water was provided ad libitum.

Semen samples collection and determination of spermatological characteristics

Semen was collected with an artificial vagina by using a teaser cow in November. Samples were taken from each bull twice a week over the course of 3 weeks (6 samples per bull). One millilitre of semen was removed from each sample for the determination of spermatological characteristics. Semen volume was determined by direct reading of the graduations markings of the collection tubes (0.5 to 15 ml). For determination of mass activity, a non-cover, slipped drop of fresh non-diluted semen was placed on a warm slide (37 ºC) and placed under a light microscope with a heated stage at 100 x magnification. The condenser diaphragm of the microscope was lowered in order to increase the contrast. The following descriptors were used for mass activity: (5) rapid dark swirls; (4) slower dark swirls and eddies; (3) slightly slower swirls; (2) no swirls, but prominent individual cell motion; (1) little individual cell motion; and (0) no individual cell motion (26). Sperm concentration was determined with a hemocytometer. Semen samples were decimally diluted with isotonic sodium citrate solution at 37 °C (3%, w/v dissolved in distilled water) to a concentration of 1:10 for the determination of initial motility. A slide was placed on a light microscope with a heated stage warmed to 37 ºC, and then a small droplet of diluted semen was placed on the slide and % motility was evaluated visually at a magnification of 400 x. Motility estimations were performed from 5 different fields in each sample. The mean of the 5 estimates was used as the final motility score. The abnormal sperm rate was determined from slides prepared with Indian ink. A total of 300 sperm cells were counted on each slide under a light microscope at 400 x magnification (27).

Samples dilution and sperm motility assay

The remainder of the semen samples of each bull were diluted to a concentration of 1:10 (semen:extender) with isotonic sodium citrate solution (3%, w/v dissolved in distilled water) + egg yolk (at the rate of 20% in the extender) extender at 37 ºC. The diluted semen samples then containing 100 x 10^6 sperm/ml were then divided into 12 sterile tubes of equal volume (0.5 ml). $PGF_{2\alpha}$ at different amounts [0 ($PGF_{2\alpha}$-free), 125, 250, 500, 1000, and 1500 µg)] was added to 6 of these tubes at 37 ºC. Additionally, different amounts of metamizol [0 (metamizol-free), 125, 250, 500, 1000, and 1500 µg)] were added to the other 6 tubes at 37 ºC. After the supplementation with $PGF_{2\alpha}$ or metamizol, all the tubes and non-diluted fresh semen were stored at 4 ºC. All samples were examined at 0, 1, 2, 4, 24, 48, 72, 96, 120, 144, 168, 192, and 216 h for sperm motility. The measurement of the motility of the diluted semen was performed in the same manner as the initial measurement of the non-diluted sperm motility.

Statistical analysis

The data are presented as mean ± SEM (standard error of means). All initial spermatological characteristics and motility of semen supplemented both with $PGF_{2\alpha}$ and metamizol were analysed with General Linear Model (GLM) procedures. The statistical models included the effect of bull, semen, $PGF_{2\alpha}$, metamizol, and time. Mean differences were determined with post-hoc LSD test. A value of $P < 0.05$ was considered statistically significant. All data were analysed with SPSS (Version 10.0).

Results

Spermatological characteristics of the bulls are given in Table 1. No significant differences in spermatological characteristics were determined between bulls and different ejaculates of the same bull ($P > 0.05$).

The motility rates (%) of non-diluted fresh semen, diluted $PGF_{2\alpha}$-free semen, and diluted semen containing the different amounts of $PGF_{2\alpha}$ are presented in Table 2. The available (50% or more) motilities continued up to 2 h for non-diluted fresh semen and 72 h for diluted semen with free $PGF_{2\alpha}$. Diluted semen with 125 and 250 µg $PGF_{2\alpha}$ added maintained available motilities up to 96 h.

While the available motility of diluted semen with 500 µg $PGF_{2\alpha}$ added was maintained for 24 h, diluted semen with 1000 and 1500 µg $PGF_{2\alpha}$ added maintained motility for 4 h. In other words, the supplementation with 125 and 250 µg $PGF_{2\alpha}$ caused a statistically

Table 1. Spermatological characteristics of the bulls.

Spermatological Characteristics							
Bull Numbers	Semen Volume (ml)	Mass Activity (0-5)	Initial Motility (%)	Sperm Concentration (….x10^6/ml)	Abnormal Sperm Rate (%)		
					Head	Tail	Total
1	3.50 ± 0.88	3.87 ± 0.23	85.25 ± 1.49	912.50 ± 86.21	3.93 ± 0.47	3.69 ± 0.54	7.62 ± 0.24
2	3.62 ± 0.42	3.75 ± 1.44	81.75 ± 1.03	988.14 ± 63.44	2.68 ± 0.85	4.37 ± 0.43	7.05 ± 0.14
3	3.32 ± 0.53	3.36 ± 0.24	85.48 ± 0.95	879.34 ± 52.92	2.63 ± 0.32	4.30 ± 0.48	6.93 ± 0.24
Means	3.48 ± 0.09	3.66 ± 0.15	84.16 ± 1.21	926.66 ± 32.20	3.08 ± 0.43	4.12 ± 0.22	7.20 ± 0.21

- Data are presented as mean ± SEM.
- No significant differences in spermatological characteristics were determined between bulls and different ejaculates of the same bull ($P > 0.05$).

significant increase ($P < 0.05$) in the motility of diluted semen stored at 4 ºC for 24 h versus diluted $PGF_{2\alpha}$-free semen. On the other hand, the supplementation with 500 and 1000 µg $PGF_{2\alpha}$ caused significant motility decreases after 4 h, while 1500 µg $PGF_{2\alpha}$ also caused a decrease after 2 h compared to diluted $PGF_{2\alpha}$-free semen ($P < 0.05$).

The motility rates (%) of non-diluted fresh bull semen, diluted metamizol-free semen, and diluted semen containing different amounts of metamizol stored at 4 ºC for 168 h are given in Table 3. The available motility of non-diluted fresh semen was maintained for up to 4 h. Although both diluted metamizol-free semen and diluted semen with 125 µg metamizol added maintained available motility by decreasing during 72 h, the available motilities of diluted semen with 250 and 500 µg metamizol continued for 48 h. However, diluted semen with 1000 and 1500 µg metamizol maintained available motility for 24 h. Metamizol supplementation with 125 and 250 µg significantly decreased the motility of diluted semen after 48 and 24 h, respectively, while the motility of diluted sperm with 500, 1000, and 1500 µg of metamizol added decreased after 4 h ($P < 0.05$) in comparison to diluted metamizol-free semen.

Discussion

In the present study, the effects of $PGF_{2\alpha}$ and metamizol on the sperm motility of bulls were investigated because of conflicting reports about the effects of PGs and possible use of PGs in the regulation of male fertility. The male and female reproductive tract is involved in $PGF_{2\alpha}$ synthesis and high concentrations of $PGF_{2\alpha}$ are existent in seminal fluid (5) and cervical mucus (2). It has been documented that intramuscular injection of $PGF_{2\alpha}$ does not affect sperm motility in dogs (16) or horses (17); however, this application of $PGF_{2\alpha}$ decreases the sperm motility of rams (18). In humans, the concentration of 19-OH- PGE is higher and 19-OH- PGF is lower in ejaculates with normal sperm motility than in those with abnormal motility (8). Gottlieb et al. (14) suggested that PGs are important regulators of sperm motility and that this effect may be mediated via effects on the ATP content in spermatozoa. One important function of seminal PGs is to stimulate the kinetic activity and motility of spermatozoa at the time of ejaculation (3). It was found that the supplementation of semen with $PGF_{2\alpha}$ increases the rate of motility in humans (11) and boars (12). In the present study, it was observed that the supplementation with 125 and 250 µg $PGF_{2\alpha}$ caused increases in the motility of diluted semen stored at 4 ºC after 24 h versus diluted $PGF_{2\alpha}$-free semen. The increase seen in sperm motility after the addition of low amounts of $PGF_{2\alpha}$ to the diluted bull semen can be explained by the direct effect of PGs on spermatozoa possibly acting on the contractile elements of the sperm, as in other tissues (3,4). The presence of high concentrations of PGF in seminal fluid is associated with poor sperm motility. (6). Post-thaw progressive motility is depressed by the addition of the salt of $PGF_{2\alpha}$ (28). Didolkar and Roychowdhury (29) have alleged that $PGF_{1\alpha}$ and $PGF_{2\alpha}$ reduced motility of sperm in vitro in humans, whereas some researchers reported that human semen $PGF_{2\alpha}$ (13,14) or the addition of $PGF_{2\alpha}$ to diluted boar semen

Table 2. The motility rates (%) of non-diluted fresh semen, diluted $PGF_{2\alpha}$-free semen, and diluted semen containing different amounts of $PGF_{2\alpha}$ over 216 h.

Groups	Time (hour)												
	0	1	2	4	24	48	72	96	120	144	168	192	216
Fresh Semen (Non-Diluted)	$82.16^{Aa} \pm 0.74$	$77.33^{Aa} \pm 0.92$	$58.16^{Ab} \pm 1.40$	$42.16^{Ac} \pm 1.03$	$2.66^{Ad} \pm 0.46$	0	-	-	-	-	-	-	-
Diluted Semen ($PGF_{2\alpha}$-free)	$75.66^{Aa} \pm 1.14$	$74.66^{Aab} \pm 0.98$	$72.33^{BCab} \pm 1.37$	$71.88^{BCab} \pm 1.65$	$62.00^{BCbc} \pm 1.80$	$56.00^{Ac} \pm 2.85$	$50.66^{Ac} \pm 2.79$	$17.33^{Ad} \pm 3.69$	$5.16^{Ae} \pm 1.80$	$1.66^{Ae} \pm 0.69$	0	-	-
Diluted Semen (with 125 µg $PGF_{2\alpha}$)	$80.53^{Aa} \pm 1.36$	$77.33^{Aab} \pm 1.23$	$75.50^{Bab} \pm 1.32$	$73.66^{Bab} \pm 1.41$	$71.33^{Bab} \pm 1.57$	$70.00^{Bab} \pm 2.06$	$66.66^{Bb} \pm 2.27$	$51.83^{Bc} \pm 4.00$	$16.66^{Bd} \pm 4.37$	$10.00^{Bde} \pm 3.39$	$8.00^{Ade} \pm 3.33$	$3.83^{Ae} \pm 1.62$	0
Diluted Semen (with 250 µg $PGF_{2\alpha}$)	$79.83^{Aa} \pm 1.11$	$79.33^{Aa} \pm 1.06$	$76.16^{Bab} \pm 1.26$	$73.83^{Bab} \pm 1.54$	$70.66^{Bab} \pm 1.54$	$69.83^{Bab} \pm 1.67$	$64.83^{Bb} \pm 2.55$	$50.50^{Bc} \pm 3.69$	$15.33^{Bd} \pm 3.06$	$9.66^{Bd} \pm 3.06$	$7.00^{Ade} \pm 2.92$	$3.66^{Ae} \pm 1.55$	0
Diluted Semen (with 500 µg $PGF_{2\alpha}$)	$77.50^{Aa} \pm 1.26$	$76.66^{Aa} \pm 1.20$	$73.16^{BCa} \pm 1.50$	$70.33^{BCab} \pm 1.77$	$57.33^{Cbc} \pm 1.43$	$46.33^{Acd} \pm 1.19$	$34.33^{Cd} \pm 1.62$	$5.33^{Ce} \pm 1.99$	0	-	-	-	-
Diluted Semen (with 1000 µg $PGF_{2\alpha}$)	$74.00^{Aa} \pm 1.25$	$72.50^{Aab} \pm 1.11$	$65.50^{ABab} \pm 1.89$	$59.66^{CDb} \pm 2.48$	$34.66^{Dc} \pm 1.90$	$14.00^{Cd} \pm 1.42$	$1.50^{De} \pm 0.42$	0	-	-	-	-	-
Diluted Semen (with 1500 µg $PGF_{2\alpha}$)	$71.33^{Aa} \pm 1.85$	$67.83^{Aa} \pm 2.00$	$59.83^{ACab} \pm 2.48$	$52.16^{ADb} \pm 1.86$	$7.50^{Ac} \pm 0.70$	0	-	-	-	-	-	-	-

- Data are presented as mean ± SEM.

-The values with different uppercases (A, B, C and D) within the same column are statistically different ($P < 0.05$).

-The values with different lowercases (a, b, c, d and e) within the same line are statistically different ($P < 0.05$).

Table 3. The motility rates (%) of non-diluted fresh semen, diluted metamizol-free semen, and diluted semen containing different amounts of metamizol over 168 h.

	Time (hour)										
Groups	0	1	2	4	24	48	72	96	120	144	168
Fresh Semen (Non-Diluted)	$79.16^{Aa} \pm 0.68$	$74.66^{Aab} \pm 0.79$	$63.50^{Abc} \pm 0.90$	$55.16^{Ac} \pm 1.13$	$6.00^{Ad} \pm 0.55$	0	-	-	-	-	-
Diluted Semen (Metamizol-free)	$78.50^{Aa} \pm 0.64$	$77.33^{Aab} \pm 0.78$	$75.16^{Aab} \pm 0.77$	$73.50^{Bab} \pm 0.83$	$71.50^{Bab} \pm 0.68$	$69.00^{Aab} \pm 0.50$	$64.66^{Ab} \pm 0.62$	$47.50^{Ac} \pm 0.74$	$30.00^{Ad} \pm 1.00$	$10.33^{e} \pm 0.53$	0
Diluted Semen (with 125 µg Metamizol)	$75.83^{Aa} \pm 0.68$	$74.83^{Aa} \pm 0.94$	$72.33^{Aab} \pm 0.88$	$68.66^{Babc} \pm 1.09$	$61.50^{BCbcd} \pm 0.68$	$57.83^{ABcd} \pm 0.61$	$50.66^{Bd} \pm 0.39$	$33.33^{Be} \pm 0.79$	$14.00^{Bf} \pm 1.40$	0	-
Diluted Semen (with 250 µg Metamizol)	$76.00^{Aa} \pm 0.90$	$75.50^{Aa} \pm 0.96$	$71.83^{Aab} \pm 0.81$	$67.50^{ABab} \pm 1.11$	$59.66^{BCbd} \pm 0.71$	$54.16^{Bcd} \pm 0.93$	$47.00^{Bd} \pm 0.85$	$19.00^{Ce} \pm 1.41$	0	-	-
Diluted Semen (with 500 µg Metamizol)	$76.33^{Aa} \pm 0.98$	$74.83^{Aa} \pm 0.97$	$69.66^{Aab} \pm 0.63$	$65.16^{ABabc} \pm 1.00$	$57.83^{Cbc} \pm 1.06$	$52.66^{Bcd} \pm 0.82$	$42.66^{BCd} \pm 0.92$	$17.33^{CDe} \pm 1.14$	0	-	-
Diluted Semen (with 1000 µg Metamizol)	$75.33^{Aa} \pm 1.04$	$74.33^{Aa} \pm 1.00$	$68.83^{Aab} \pm 0.61$	$65.16^{ABab} \pm 0.94$	$55.66^{Cbc} \pm 1.11$	$45.83^{BCc} \pm 1.22$	$31.66^{CDd} \pm 1.38$	$6.50^{De} \pm 0.68$	0	-	-
Diluted Semen (with 1500 µg Metamizol)	$74.50^{Aa} \pm 0.96$	$73.33^{Aa} \pm 0.93$	$67.83^{Aa} \pm 0.61$	$63.33^{ABab} \pm 0.90$	$53.33^{Cb} \pm 0.93$	$36.00^{Cc} \pm 1.66$	$19.66^{Dd} \pm 1.28$	0	-	-	

- Data are presented as mean ± SEM.

-The values with different uppercases (A, B, C and D) within the same column are statistically different ($P < 0.05$).

-The values with different lowercases (a, b, c, d, e and f) within the same line are statistically different ($P < 0.05$).

(15) does not affect sperm motility. On the other hand, Cohen et al. (9) determined that when $PGF_{2\alpha}$ is added in greater than physiological concentrations, it can also reduce sperm motility in vitro in humans, and also suggested that $PGF_{2\alpha}$ stimulates the production of cyclic GMP, a substance that has been shown to markedly reduce sperm motility. Fayed (10) has also reported that in vitro supplementation with high levels of $PGF_{2\alpha}$ (300, 600 and 1200 µg/ml) to diluted bull epididymal spermatozoa suppressed motility and even induced sperm membrane damage and permeability. It is clearly seen that sperm motility is related to the concentration of $PGF_{2\alpha}$. In this study, sperm supplementation with 500, 1000, and 1500 µg $PGF_{2\alpha}$ caused significant decreases in motility. The decreases in sperm motility induced by high amounts of $PGF_{2\alpha}$ noted in the present study are consistent with the findings of other investigators. These decreases may be explained by the stimulation of production of cyclic GMP by $PGF_{2\alpha}$.

Metamizol is a highly potent analgesic and antipyretic used especially in the treatment of pain and hyperthermia (20). Metamizol and other anti-inflammatory substances are potent inhibitors of cyclooxygenase, which mediates prostaglandin biosynthesis (23). The motility of spermatozoa is dependent on the presence of certain amounts of PGs, which have a protective function on sperm motility, possibly acting as cytoprotective agents (1). Therefore, inhibition of this enzyme prevents biosynthesis of PGs (4,22,23), and a rapid fall in sperm motility consequently occurs (17,21). Tanyıldızı and Bozkurt (25) observed that intramuscular administration of metamizol and oral intake of acetylsalicylic acid twice daily for 4 days caused an increase in motility of ram semen. Conte et al. (24) observed that when seminal PG levels are high, PG inhibition by indomethacin will cause a significant improvement in sperm count and motility, but not normal-low seminal PG levels. Acetylsalicylic acid reduces sperm count and percentage of motility, and causes alterations in the morphological characteristics of spermatozoa by affecting the epididymal milieu in post-pubertal rats (30). On the other hand, it has been reported that intramuscular administration of indomethacin (3, 6, and 9 mg/kg) has no effect on motility or other spermatological features in rams (18). In the present study, sperm supplementation with different amounts of metamizol decreased the motility of diluted semen stored at 4 °C in comparison to diluted metamizol-free semen. The findings of this study are compatible with the in vivo results of some researchers that have investigated metamizol and other substances, while they are not compatible with others. This situation may be dependent on in vitro use and the amounts of metamizol, and the use of different animal species.

Metamizol also has a significant inhibiting effect on the activity of the glucose 6-phosphate dehydrogenase enzyme, which catalyses the first step of the pentose phosphate metabolic pathway, both in vivo and in vitro. (31). The pentose phosphate metabolic pathway is a unique source of NADPH and NAD^+, of which the addition and/or presence as an energy source could prevent the time-dependent decrease of motility (6). Consequently, inactivation of glucose 6-phosphate dehydrogenase by metamizol may cause a reduction in sperm motility. In the present study, a time-dependent decrease was observed in the motility of bull semen after supplementation with different amounts of metamizol, and this may be explained by the inhibition of cyclooxygenase and/or glucose 6-phosphate dehydrogenase.

In conclusion, the addition of small amounts of $PGF_{2\alpha}$ to diluted bull semen that contained 100 x 10^6 sperm/ml, but not metamizol or large amounts of $PGF_{2\alpha}$, helped maintain motility during short-term storage at 4 °C. Therefore, sperm could be supplemented with small amounts of $PGF_{2\alpha}$, which may be effective for improving sperm motility in bulls.

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