

An *in vitro* study on the effect of bradykinin and bradykinin analogous on bull's spermatozoa motility

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Abstract.

Administration of bradykinin, a peptide compound belonging to the kallikrein - kinin system, to bull's semen was found to activate spermatozoa motility two and three hours following treatment. In contrast, sperms motility was remarkably reduced when bradykinin analogous B3820, B3824 and B3878, were added to semens compared to control samples, consisting of diluted semens only. These results suggest the presence of specific receptors for bradykinin on spermatozoa plasma membrane, and that the analogous used exhibited antagonists effects of bradykinin.

Key words. Bradykinin, bradykinin analogous, spermatozoa, motility.

Introduction

Among the biologically active peptides developed by mammals' blood, we found bradykinin, a peptide belonging to the kallikrein-kinin system. Some of the physiological action of this peptide are now well documented such as its action on blood vessels, smooth muscles contractions, and its effect on capillary permeability (1). Yet, the possible implication of bradykinin in

reproductive physiology of mammals has become interesting over the years. For instance, ovulation was found to coincided with a marked fall in plasma bradykininogens in both rats and women (2). The mode of action of the kallikrein-kinin system has been assumed to be mediated through the activation of two distinct plasma membrane receptors known as B₁ and

B₂, found in various types of tissues (3-6). Because the kallikrein-kinin system has long been assumed to, somehow, affect the reproductive axis of mammals such as increasing spermatozoa activity and provoking muscle contraction of the uterus (7-9), we tend, through the present investigation, to assess the effect of bradykinin and some of its analogous on bull's spermatozoa activity.

Materials and methods

Peptides. Bradykinin was obtained from Sandoz (Switzerland), while bradykinin analogous were supplied by Vavrek Reimond, from the university of Colorado, USA.

The biochemical structure and the subsequent molecular weight (M.W) of each peptide is as follows :

Bradykinin : Arg - pro - pro - gly - phe -
ser - pro - phe - Arg
(M.W. 1240)

B 3820 : Arg - pro - Hyp - gly - ty - ser - D phe
- Ty - Arg.TFA
(M.W. 1780)

B 3824 : D Arg - Arg - pro - Hyp - gly - Ty -
ser - D phe - Ty - Arg.TFA
(M.W. 1980)

B 3878 : lys - lys - Arg - pro - pro - gly - Ty -
ser - D phe - Ty - Arg.TFA
(M.W. 2350)

Where : Ty - beta / 2 - tyenil / L -
alanin

Hyp : 1.4 - hydroxyprolin

TFA : tri - fluoro - acetic acid.

Semen samples and experimental design. Semen samples were obtained from mature bulls using artificial vagina. Only samples with good biological characteristics had been used in the present study, before being diluted in 2.8% sodium citrate (1/1V).

The diluted semen samples were randomly divided into five groups each comprising 18 samples as follows :

Group A, comprising diluted semen samples only to serve as controls.

Group B, consisted of diluted samples plus bradykinin at 10^{-9} M (1 : 1V).

Group C, D and E, each consisting of diluted semen samples plus one of bradykinin analogous at 10^{-7} M (1 : 1V).

Following an incubation period of four hours at 37°C , the percentage of spermatozoa motility was estimated throughout the four successive hours using the counting slide of Burker.

Statistical analysis. Data were analyzed using analysis of variance to compare mean values within groups, while comparisons between means of different groups were made using student's t-test (10).

Results

The effect of bradykinin administration on spermatozoa motility. Figure 1 illustrates the effect of bradykinin administration on the motility of bull's spermatozoa.

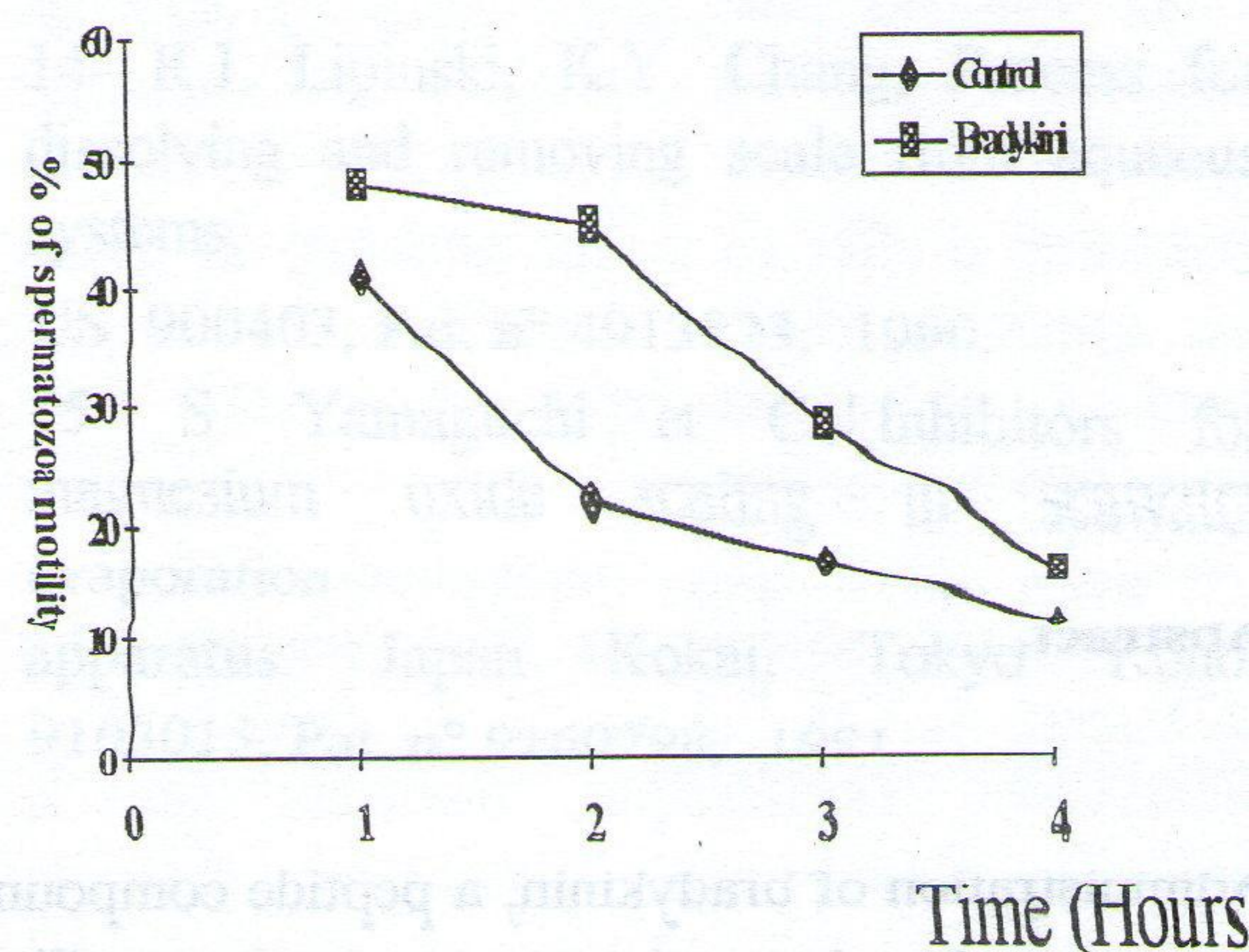


Fig. 1 Changes in spermatozoa motility (%) of bull's semens, either diluted in sodium citrate as controls (A) or, receiving bradykinin (B). (Points are represented as mean $\pm$ SEM ; n = 18).

Following the first hour of treatment, there was a 17% increase in sperms motility compared to control semen samples. Two hours after treatment such motility was considerably reduced ($P < 0.005$) in control samples, while that of bradykinin treated samples was still high and remarkably greater ($P < 0.005$) than that recorded in controls. Spermatozoa motility was much elevated ($P < 0.005$) at hour three and four of the experiment in bradykinin treated semens, compared to control samples, where the increase in the percentage of motility was estimated to be 60% and 35% respectively.

The effect of bradykinin analogous administration on spermatozoa motility. The effect of administering bradykinin analogous on the motility of bull's spermatozoa is shown in figure 2.

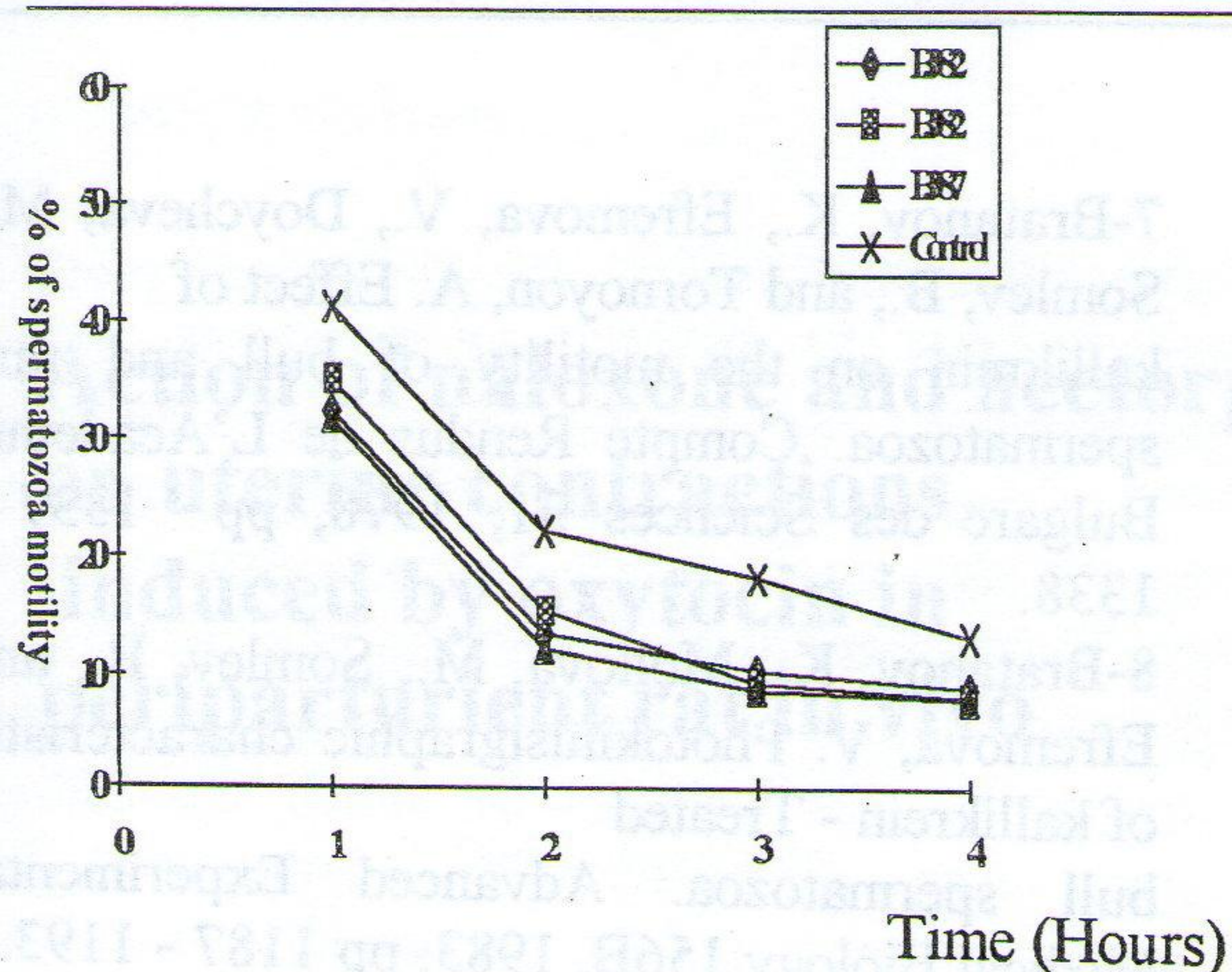


Fig. 2 Changes in spermatozoa motility (%) of bull's semens diluted in sodium citrate as controls (A), receiving the bradykinin analogous B3820 (C), the bradykinin analogous B3824, and in those receiving the bradykinin analogous B3878. Each point represents mean $\pm$ SEM ($n = 18$ in each group).

Spermatozoa motility was significantly suppressed ($P < 0.005$) in controls at hour two of the experiment, and was even much reduced ($P < 0.001$) after the fourth hour. In contrast, all the used bradykinin analogous had a depressing effect on sperms motility that was significantly ($P < 0.05$) reduced after one hour of treatment, compared to that of controls (Fig.2). The inhibitory effect of the utilized bradykinin analogous was enhanced ($P < 0.001$) after the third and fourth hour of treatment compared to control samples, consisting of diluted semens only.

Discussion

The kallikrein - kinin system has long been known for its involvement in reproductive physiology of mammals. Most, if not all, investigations agree that the system interferes deeply in the activation of spermatozoa motility, even though the mechanism (s) by which such a system affect sperms motility is still far from being understood (7-9). Results arising from these experiments demonstrate that bradykinin does not only activate spermatozoa motility *in vitro*, but it also maintains such an activity for a longer period when added at a concentration of

10^{-9} M. After one hour of bradykinin administration, spermatozoa motility did not undergo any significant decrease, but it remained, in stead, twice as high as that of control samples (Fig.1). Furthermore, the decrease in spermatozoa motility in bradykinin treated samples was not as low as that recorded in controls, following the remaining time of the experiment

Although the mechanism underlying the mode of action of bradykinin on sperms motility is still not well established, it seems more likely that the peptide activate a cascade of biochemical reactions in the system regulating sperms movements, after adhering to specific spermatozoa membrane receptors (3).

On the other hand, all the bradykinin analogous utilized in the present study showed inhibitory effects on sperms motility and, thus, conveying an antagonist action of bradykinin. Spermatozoa motility was slightly reduced in the samples receiving B 3820, B 3824 and B 3878, compared to controls consisting of diluted semen samples only, after one hour of their administration. Yet, the suppressing action of these three substances was apparent at hour two of the experiment and to a greater extent throughout the remaining time of the experiment (Fig.2). The antagonist nature of some of these analogous has been reported in other physiological events. For instance, B 3824 has been found to be a bradykinin antagonist in terms of its effect on blood pressure in rats (11). A similar conclusion has been drawn by Schachter and others (12) in that B 3824 has an opposit effect to bradykinin on vessels permeability in rabbits.

To conclude with, even though the results provide some clear evidences for that bradykinin activates and maintains sperms motility and that the analogous used have an inhibitory effect and serve as bradykinin antagonists, future experiments would be of great interest, particularly any designed work investigating the mode of action of bradykinin on sperms motility. Besides, it seems also interesting to see whether there is a dose dependent effect of bradykinin on spermatozoa motility or not.

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