Progesterone Increases the Progressive Motility of Human Sperm

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Abstract

Sperm are transcriptionally inactive. Transformation from immature to mature sperm capable of fertilizing an egg depends on altering the proteome. For example, proteomic studies identified receptors not predicted to be expressed on the sperm surface, notably the progesterone receptor.

This discovery raises the question of whether progesterone exerts nongenomic effects on human spermatozoa. Here we aimed to answer this question. Sperm from a normal individual were subjected to Percoll gradient centrifugation and treated with different concentrations of progesterone for 2 h at 37°C.

Sperm motilities were determined using a Neubauer chamber according to the World Health Organization's standards. We measured the percentage changes in motility variables as follows: fast and straight progressive motility, slow progressive motility, nonprogressive motility, and nonmotility.

Progesterone (250 ng/ml) increased sperm motility by 55.96% compared with the control without added progesterone (22.6%). Significant concentration-dependent increases in fast and progressive motility reached 29.55% compared with the control (10.1%).

Progesterone (250 mg/ml) reduced the percentage of nonmotile cells to 44% compared with the control (77.4%). Progesterone increased the motility and progressive motility of human sperm in a concentration-dependent manner.

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Introduction

Progesterone mainly functions in the female reproductive cycle.[1] In spermatozoa, progesterone regulates the motility required to fertilize an ovum.[1,2] Progesterone is expressed mainly by cumulus oophorus cells in the follicular fluid.[1-3] Subsequent to ejaculation, spermatozoa enter oophorus cells and the fluid containing prostasomes in a pH-dependent manner.[4] Capacitation, which occurs when sperm reach endometrial cells,[5] is required to prepare spermatozoa to undergo the acrosome reaction that occurs in the uterus or fallopian tubes.[3] This process induces hyperactivated motility, which is a specific and progressive type of motility of spermatozoa.[3,5] This motility, characterized by irregular movements of the head and flagellum of a spermatozoon, is required to penetrate an ovum.

An insufficient amount of progesterone is associated with infertility.[3,6] Progesterone mobilizes the Ca^{2+} present in the sperm, thereby generating Ca^{2+} release. This process generates a biphasic increase, which mediates a progesterone-induced Ca^{2+} influx in human sperm.[6] The absence of detectable progesterone in spermatozoa can cause asthenozoospermia, which reduces the motility of sperm and is associated with infertility.[6] Here we show that progesterone increased the motility and progressive motility of sperm in aconcentration-dependent manner.

Methods

Reagents. Unless otherwise stated, all the chemicals used in this study were purchased from Sigma (USA). Biggers, Whitten, and Whittingham (BWW) stock solution contained 5.54 g of NaCl, 0.356 g of KCl, and 0.25 g of $CaCl_2.2H_2O$; to this, 0.162 mg of K_3PO_4 and

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0.294 g of $MgSO_4.7H_2O$ were added to a final volume of 1 l of Milli-Q water. A 2-fold concentrated BWW stock solution was stored at 4°C for 3 months before use. The BWW working solution was prepared by adding 200 ml of BWW stock to 0.42 g sodium hydrogen carbonate, 0.2 g glucose, 0.006 g sodium pyruvate, 0.2 g polyvinyl alcohol (PVA), 0.74 g sodium lactate, 2 ml penicillin–streptomycin, and 4 ml HEPES buffer (Gibco, USA). PVA can be substituted with 0.6 g albumin bovine fraction V. Percoll (100%) contained 0.1 g of PVA, 0.21 g of $NaHCO_3$, 10 ml of 10× HAMS/F10 medium, 90 ml of Percoll, 0.05 ml of sodium pyruvate stock, and 0.37 ml of sodium lactate. Spermatozoa diluting fluid was prepared by mixing 50 g of sodium hydrogen carbonate and 10 ml of formaldehyde. The eosin solution contained 500 mg of eosin and 0.09 M NaCl in 10 ml of Milli-Q water. The Percoll gradient was prepared using 3 ml of 50% Percoll in a Falcon tube, which was underlaid with 3 ml of 100% Percoll and stored at 37°C.

Sperm samples. Procedure for sperm donor collection had been approved by ethical committee at the Faculty of Medicine, Universitas Indonesia (238/UN2.F1/ETIK/2017). Sperm samples were obtained from a normal healthy donor who did not engage in sexual activities for 2 days preceding sample collection. The sperm samples were then subjected to Percoll gradient centrifugation. Briefly, undiluted sample was transferred into a percol gradient containing BWW (50/100%). The mixture was then centrifuged at 2,500 rpm for 30 min to separate sperm cells from seminal plasma. Supernatant was discarded and pellet was re-suspended in 3 mL BWW. Mixture was then centrifuge at 2,500 rpm for 15 minutes. Supernatan was discarded again and pellet was re-suspended in 1 mL BWW.

Sperm count analysis and treatment. 5 µl of undiluted sample was added into 95 uL sperm diluting fluid briefly vortexed. To determine the density of the sperm, 10 µl of diluted sample was applied onto hemocytometer and let it settle for 2-3 minutes. Under light microscope, sperm were counted on 5 squares at 40× magnification. The optimum number of a spermatozoa sample for treatment was 10 x 10^6 cells for each treatment group. Sperm cell were divided into 5 treatment groups which are 0 ng/ml (as a control), 250, 500, 750, 1000 ng/ml. The tube containing sperm cells with progesterone were then incubated at 37 C for 2 hours. Motility of the sperm after treatment was assessed according to the WHO standard using Neubauer chamber. Four categories of sperm motility were examined namely the percentage of motile, progressive motility, slow motility and static (no movement was observed). The analysis was repeated 4 times.

Results and Discussion

Progesterone (500 ng/ml) significantly increased the motility of the sperm (Figure 1). Compared with the control (22.6%), the percentages of motile sperm in the progesterone-treated samples were higher, ranging from 40.47% to 55.96%, while the percentage of nonmotile spermatozoa decreased from 77.4% to 44%–59.52% (Table 1). These data show that there was a significant difference in sperm motility ($p<0.05$) between progesterone-treated and untreated samples. However, at higher concentrations of progesterone, the differences were not significant. These data indicate that the effect of progesterone on sperm motility was concentration-dependent.

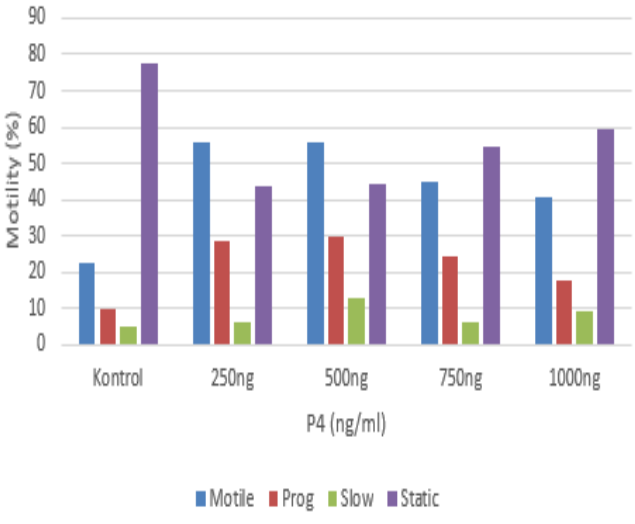

Figure 1. The effects of progesterone on sperm motility. Motility, blue; progressive motility, red; slow motility, green; and nonmotility, purple.

	Motility					
No	Parameter	Control (%)	250ng (%)	500ng (%)	750 (%)	1000ng (%)
1	Motile	22.6	55.96	55.87	45.2	40.47
2	Prog	10.1	28.32	29.55	24.17	17.65
3	slow	5.3	5.95	13.1	5.95	9.13
4	static	77.4	44	44.12	54.8	59.52

Table 1. Mean percentage motilities of spermatozoa

The effects of progesterone on human spermatozoa Progesterone affects the motility of spermatozoa as follows: inducing the acrosomal reaction, binding to the zona pellucida, hyper-activating motility, and mediating the penetration of the oolemma.[7-9] For example, transient progesterone-induced Ca^{2+} signaling[10] in spermatozoa, particularly in the neck, contributes to motility.[10] Further, Ca^{2+} signaling involves the activation of the metabotropic reaction, which is characterized by a temporary inactivation of the cellular response.[9] Moreover, progesterone stimulates the increase in Ca^{2+} concentration.[11,12] Ca^{2+} is then mobilized by the activation of the cyclic ADP ribose (cADPR) ryanodine receptor[11] that resides on the endoplasmic and sarco-plasmic reticula.[10]

In mature spermatozoa, the redundant nuclear envelope within the neck of spermatozoa mediates the release and reuptake of Ca^{2+}.[11-13] The influx of Ca^{2+} hyperactivates spermatozoa to enable fertilization through cation channel sperm-associated (CatSper) ligand-gated ion channels.[11,14] CatSper channels mediate alterations in the oscillations of Ca^{2+} concentrations.[15] CatSper resides in the flagella of spermatozoa and regulates their chem..otactic response.[15] CatSper is activated directly by progesterone and is sensitive to internal pH.[16] In response to progesterone, the CD38 molecule is translocated from membranous vesicles (prostasomes) secreted by the prostate gland prostasomes, which mediate the production of cADPR and Ca^{2+} signaling.[10] Therefore, our present data indicate that pro-gesterone increases the motility of spermatozoa. However, to our knowledge, the role of CatSper in human spermatozoa is not established.[17] Evidence indicating the involvement of CatSper and the species specificity of ion channels does not allow definitive conclusions to be drawn between the associations of progesterone, CatSper, and the motility of spermatozoa.[18] Nevertheless, investigators agree that progesterone acts through a nongenomic signaling mechanism to induce Ca^{2+} and its sustained signaling.[19-21]

Conclusions

Progesterone, at concentrations ranging from 250 to 500 ng/ml, increases sperm motility. Progressive motility was also increased in the presence of 500 ng/ml progesterone. Further studies are required to identify the molecular mechanism underlying this phenomenon.

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Declaration of Interest

The authors report no conflict of interest.

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