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Progesterone decrease plasma membrane in human sperm with subnormal hypoosmotic swelling test scores

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ABSTRACT

BACKGROUND
Progesterone (P4) is known as a female hormone affecting oocyte maturation and developing uterine wall. A proteomic study identified several receptors including P4 receptors on human sperm. The role of P4 in human sperm cells remains unknown as to whether P4 has non-genomic effects on human sperm. The present study aims to determine the effect of progesterone (P4) on the hyperactivated motility and membrane integrity of human sperm cells.

METHODS
Semen from normal individuals was obtained from donors. The semen was washed by gradient density centrifugation. P4 was added to each semen sample to final concentrations of 0 (control), 250, 500, 750 and 1000 ng/mL. After the sample treatment was completed, the sperm membrane integrity was assessed with the hypoosmotic swelling test (sodium citrate dihydrate and D-fructose) and the hyperactivated sperm motility parameter was determined with the Computer Assisted Sperm Analyzer [CASA] (Hamilton Thorne, IVOS II, USA). The percentage was then compared between the treatment groups and the control group. The percentage differences were analyzed with the Sigmastat version 2.0 statistical program.

RESULTS
Administration of P4 increased sperm hyperactivated motility when compared with the control group at a concentration of 500 ng/mL, but the increase was statistically not signicant ($p>0.05$). In contrast, P4 decreased sperm membrane integrity significantly ($p=0.042$). And the mean of plasma membrane integrity in all groups was subnormal hypoosmotic swelling test score.

CONCLUSION
Progesterone administration tends to increase sperm hyperactivated motility. The integrity of plasma sperm membrane was affected by progesterone.

Keywords: Progesterone, fresh human sperm,membrane integrity, motility

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INTRODUCTION

Progesterone (P4) is a steroid hormone which is released by the cumulus cells surrounding the ovum.[1] Ovulation, implantation, and pregnancy are processes that involve P4.[2]This steroid hormone causes the sperm cells to move towards the ovum then helps them to penetrate into the egg.[1] Calcium influx and chloride efflux are induced by P4 as an essential way for successful fertilization, with effects that include sperm hyperactivation, acrosome reaction, and chemotaxis towards the ovum.[1,3] The latter is described as irregular movement of the sperm head and flagellum to penetrate the ovum. One study reports that an insufficient amount of P4 is related to infertility,[3] which may be due to the fact that P4 is a calcium mobilizing agent for sperm by generating Ca^{2+} release, causing a biphasic increase in Ca^{2+}, and mediating P4-induced Ca^{2+} influx in human sperm. Lack of P4 in sperm causes asthenozoospermia and is highly related to infertility problems.[3,4]

The human sperm proteome study by Baker et al. [5] proved that sperm have progesterone receptors on their plasma membrane,[5] and that there are several complex arrays of receptors including extragenomic P4 receptors.

The study by Sayme et al.[6] showed that P4 increased hyperactivated motility in human sperm cryopreserved by slow freezing and vitrification, at P4 concentrations of 0, 25, 50 nM. The present study was different from that of Sayme et al.[6] in that our study used fresh human sperm. The aim of the present study was to evaluate the effect of various P4 concentrations on hyperactivated motility and membrane integrity of fresh human sperm.

METHODS

Research design

This was an experimental in vitro study using normozoospermia donor semen at the Molecular Biology Laboratory, Universitas Indonesia, over a period of 1 year from March 2017 to March 2018.

Semen samples

This study used semen samples obtained from donors by masturbation after abstinence for 3-5 days. The samples were divided into five groups, comprising one group without P4 as control and four groups with different doses of P4 (250, 500, 750, 1000 ng/mL). Each group contained a total of 10 million sperm. The experiment was repeated nine times using different semen donors.

Sperm preparation and intervention

Sperm was obtained from healthy normal donors. The sperm was washed out from the seminal plasma by putting 3 mL of the sperm sample on top of a Percoll gradient and centrifuging it at 1900 rpm for 30 minutes. After centrifugation, the sperm was collected and separated from the supernatant. Then the sperm pellet was put into 1 mL of warm Biggers-Whitten-Whittingham (BWW) medium. This medium consists of 0.21 g sodium hydrogen carbonate, 0.1 g D-glucose, 0.1 g polyvinyl alcohol (PVA), 0.05 ml sodium pyruvate stock, 2 ml 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 0.37 mLsodium lactate. After that, the sample was checked to determine its normality. A volume of 95µL sperm was added to 5 µL sperm diluting fluid, which is made from 2.5 g sodium hydrogen carbonate, 0.5 ml formaldehyde, and 50 ml H_2O. A volume of 10 µL diluted sample was added to a hemocytometer, left to stand for 2-3 minutes, then the number of sperm cells was counted in the five squares of the hemocytometer at 40x magnification. If the sample was normal, the sperm was incubated with progesterone (P4). Five hundred µL aliquots of the sperm sample, containing $10x10^6$sperm cells, were put into five Eppendorf tubes. Then P4 was added into those five tubes, at concentrations of 0, 250, 500, 750, and 1000 ng/mL, respectively. After that, the samples were incubated at 37ºC

for 2 h. before being subjected to membrane integrity and motility testing.

Measurement of sperm hyperactivated motility

The evaluation of sperm concentration and motility was performed using the Computer Assisted Sperm Analyzer [CASA] (Hamilton Thorne, IVOS II, USA). Sperm hyperactivated motility measures include total motility (TM) (%), forward progressive motility (FPM) (%), and velocity parameters such as curvilinear velocity (VCL) (m/sec), straight-line velocity (VSL, µm/s), linearity of forward progression, which is the VSL to VCL ratio (linearity coefficient, LIN, %), amplitude of sperm cell lateral deviation about its axis of progression or average path (amplitude of lateral head displacement, ALH, µm).[7]

Measurement of sperm membrane integrity

Sperm membrane integrity was assessed using the hypo-osmotic swelling (HOS) test.[8] Ten microliters of sperm were mixed with 100 µL of HOS solution. Tail morphology was evaluated under a light microscope at 400x magnification. Sperm cells with bended/curly tails were considered as sperm with an intact plasma membrane, whereas those with straight tails were regarded as sperm with a disrupted plasma membrane. The percentages of intact and disrupted sperm plasma membranes were obtained by counting a total of 100 curly and straight-tailed sperm cells. According to WHO, the membrane integrity of normal sperm is more than or equal to 58% (5th centile, 95% CI 55–63). [9]

Statistical analysis

Mean values of movement parameters and labeled sperm were compared between control and test samples by one way Anova. The post hoc differences between groups were analyzed by the Tukey HSD test. A value of $p< 0.05$ was considered statistically significant.

Ethical Clearance

This research has been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia, under No. 303/UN2.F1/ETIK/III/2018.

RESULTS

The P4 dose-dependent effects on sperm motility and progressivity are illustrated in Table 1. In group I (control group), the percentage of motile sperm during the observation period was 30.14 ± 16.25%. The control group had a lower percentage of motile sperm compared to the P4 treatment groups. The highest percentage of motile sperm was in the P4 250 ng/mL group, amounting to 36.03 ± 17.69%. There were no significant differences between the control group and the P4 treatment groups ($p>0.05$).

In the control group, the percentage of progressive sperm during the observation period was 10.02 ± 9.01%. The control group showed a lower percentage of progressive sperm compared to that observed in the P4 treatment groups, but the result was not statistically significant. The highest percentage of progressive sperm was in the P4 500 ng/mL group, namely 15.46 ± 15.27%.

Table 1. The percentages of motile sperm and progressive movement parameter in the control group and after incubation with progesterone

Parameter	Progesterone concentration (ng/mL)					p value
	I	II	III	IV	V	
Motile (%)	30.14 ± 16.25	36.03 ± 17.69	35.32 ± 23.95	32.02 ± 21.27	33.57 ± 6.88	0.173
Progressive (%)	10.02 ± 9.01	12.20 ± 10.98	15.46 ± 15.27	14.11 ±13.64	13.20 ± 9.79	0.216

I: control; II: incubation with progesterone 250 ng/mL; III: incubation with progesterone 500ng/mL; IV: incubation with progesterone 750ng/mL; V: incubation with progesterone 1000 ng/mL. Values are presented as mean ± SD

Table 2. Sperm motion parameter and membrane integrity in the control group and after incubation with progesterone

Parameter	Progesterone concentration (ng/mL)					p value
	I	II	III	IV	V	
VCL (µm/sec)	52.13 ± 12.12	59.18 ± 8.73	61.03 ± 15.81	60.75 ± 23.17	62.26 ± 9.59	0.074
LIN (%)	40.10 ± 8.06	43.6 ± 6.82	43.49 ± 6.41	43.25 ± 6.30	42.22 ± 5.12	0.113
ALH (µm)	3.00 ± 0.82	3.62 ± 0.70	3.49 ± 0.85	3.54 ± 1.09	3.63 ± 0.84	0.126
Membrane Integrity (µm/sec)	43.00 ± 1.15	34.00 ± 1.00	37.00 ± 3.00	44.00 ± 15.00	49.00 ± 16.00	0.042

I: control; II: incubation with progesterone 250 ng/mL;III: incubation with progesterone 500ng/mL; IV: incubation with progesterone 750ng/mL; V: incubation with progesterone 1000 ng/mL Values are presented as mean ± SD; p= significance level, p<0.05. VCL: curvilinear velocity; LIN: linearity coefficient; ALH: amplitude of head lateral displacement

After 2 h of P4 incubation, VCL in the control group (52.13 ± 12.12 µm/sec) was observed to be lower compared with the P4 treatment groups (59.18 ± 8.73 µm/sec - 62.26 ± 9,59 µm/sec) (p=0.074), but this result was not statistically significant (p>0.05). The LIN in the control group (40.10 ± 8.06%) was lower than that in the P4 treatment groups (>42%) (p=0.113). The amplitude of the head lateral displacement in the control group (3.00 ± 0.82 µm) was lower than in the P4 treatment groups (>3 µm). Furthermore, no statistical differences were found for ALH at any P4 dose (Table 1).

In all groups, the mean of the plasma membrane integrity had a subnormal hypo-osmotic swelling test (HOST) scores (<50%). P4 addition to sperm slightly decreased membrane integrity of the sperm cells in the P4 250 ng/mL and 500 ng/mL groups but increased sperm membrane integrity in the P4 750 ng/mL and 1000 ng/mL groups compared to untreated sperm (p=0.042; Table 2), the increase being statistically significant. A posthoc Tukey test showed that the control group and P4 treatment groups at doses of 250, 500, and 750 ng/mL differed significantly at p<0.05, with overall mean differences of respectively -3.03, -4.18 and 2.71; the control group was not significantly different from the P4 1000 ng/mL group (Table 3).

DISCUSSION

The P4 treatment samples had different motile sperm percentages even though all fulfilled normozoospermia criteria (motile sperm percentage >32%).[10,11] In contrast to progressive sperm percentages, the minimum semen criteria were achieved after P4 500 ng/mL treatment. This data indicates that P4 increases the percentage of progressive and motile sperm. Progesterone is a steroid hormone with a non-genomic action that causes increased intracellular Ca^{2+} to trigger sperm motility and hyperactivation, and acrosome reaction.[12] Therefore it is expected that the addition of an optimum dose of P4 can exert potential effects on sperm motility.

The control group of this study showed the lowest hyperactivity of sperm compared with after P4 treatment. This is consistent with a previous study by Sumigama et al.[13] who reported that fertilization increased when the sperm were exposed to a medium containing P4 compared to the control (BWW medium). This was because the administration of P4 stimulated an increased Ca^{2+} concentration in the sperm.

Table 3. Plasma sperm membrane integrity with Tukey HSD post-hoc comparison

Treatment groups		Mean Difference	p value
I	II	- 3.0333	0.015
	III	- 4.186	0.002
	IV	2.710	0.026
	V	0.930	0.392
II	III	-1.153	0.293
	IV	5.743	0.000
	V	3.963	0.003
III	IV	6.896	0.000
	V	5.116	0.001
IV	V	-1.780	0.017

Note : mean difference is significant at the 0.05 level

The elevation of intracellular Ca^{2+} from ionic influx and detachment from the intracellular reserve is very important in sperm function, resulting in changes in sperm motility and lipid membrane remodeling which causes the sperm to form bonds with the pellucid zone and induce acrosomal reactions.[4,14] Curvilinear velocity, LIN and ALH are important indicators of hyperactivity for successful fertilization, so it can be concluded that P4 can be a mediator for in vitro fertilization.[15,16]

Based on Table 2, it can be seen that sperm membrane integrity has slightly decreased for 2 h incubation, based on WHO criteria (normal ≥58%). To determine whether there was a significant difference in sperm membrane integrity in each P4 treatment group, statistical analysis was performed. Samples from two donors had different percentages between the control and experimental groups.

Lodi et al.[17] demonstrated that there is a relationship between motility, viability and membrane integrity. However, this is not in accordance with our results. The study of Duru et al.[18] revealed that P4 has no effect on the HOS test to improve sperm membrane integrity after postfreezing. This is also supported by research by Smith et al.[19] showing that P4 can disrupt the integrity of the sperm membrane. Post-thaw sperm cells are alive but have low membrane integrity.

The integrity of the sperm membrane is determined by several factors. Henning et al.[20] reported that washing by density gradient centrifugation can cause sperm membrane integrity disruption due to the several stages of the washing process that can cause sperm cell membrane damage. Mohammadi and Mahdion [21] stated that the sperm membrane is biochemically disrupted during the process of freezing and thawing.

In the present study, it appears that the P4 treatment has an important effect, but perhaps the study has an inadequate number of samples for this difference to be statistically significant. Another limitation is that semen donor current disease was underreported, which may have caused an underestimation of the true association. Our study was able to demonstrate that P4 in vitro increased the hyperactivated motility in sperm cells, and this can be used to improve the success rate of intra-uterine insemination.[22] Next, a study on time-dependent P4 effects should be carried out to determine the time required for sperm incubation with P4.

CONCLUSIONS

The addition of progesterone in vitro tends to increase sperm motility, but the increase is statistically not significant. Plasma sperm membrane integrity decreasesd so that progesterone have an effect on sperm membrane integrity.

CONFLICT OF INTEREST

Competing interests: no relevant disclosures.

FUNDING DISCLOSURE

There is no financial conflict of interest to disclose.

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CONTRIBUTORS

SS and DAP were responsible for managing research designs, collected

manuscripts and funding. SS contributed to data acquisition and LY to data analysis. All authors have read and approved the final manuscript.

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