In Vitro Human Sperm Activation by Using Progestrone Medroxy-Acetate

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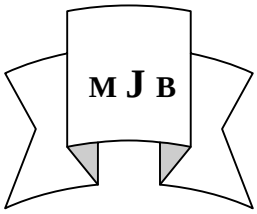

Abstract:

The aim of this study is investigate the effect of incubation periods (90 and 120 minutes) on the sperm functions of ten human asthenospermic specimens which are incubated with different concentrations of progestrone medroxy-acetate (0.1 , 0.4 and 1 mg/ml). These concentrations were added to glucosaline medium (control) and used for in vitro sperm activation .

The incubation of asthenospermic specimens with glucosaline supplemented with 0.4 mg/ml progestrone medroxy – acetate caused a significant increase ($p < 0.001$) in sperm motility percent and grade activity compared to control group (glucosaline alone) after 90 and 120 minutes . So progestrone medroxy-acetate, when used at 0.4 mg / ml can maintain the improved sperm motion for longer duration enough to increase the number of sperm reaching the upper reproductive tract and increase the fertilization capacity in the assisted reproduction technique.

الخلاصة:

تهدف الدراسة الحالية إلى التحري عن تأثير فترتي الحضن 90 و 120 دقيقة على وظائف النطف البشرية الواهنة والتي حضنت مع تراكيز مختلفة من خلات الميدروكسي بروجسترون (0.1 ، 0.4 ، 1 ملغم / مل). أضيفت تلك التراكيز إلى المحلول الفسيولوجي السكري الذي مثل بمفرده مجموعة السيطرة واستخدمها لتنشيط النطف خارج الجسم .

سبب حضن النطف الواهنة مع المحلول الفسيولوجي السكري المجهز بـ 0.4 ملغم / مل من خلات الميدروكسي بروجسترون زيادة معنوية ($p < 0.001$) في النسبة المئوية للنطف المتحركة ودرجة نشاط النطف مقارنة مع مجموعة السيطرة بعد 90 و 120 دقيقة من المعاملة . لذا يعد استخدام 0.4 ملغم / مل من خلات الميدروكسي بروجسترون وسيلة للحفاظ على الزيادة في حركة النطف لفترة طويلة تكون كافية لزيادة عدد النطف الواصلة إلى القناة التناسلية العليا وزيادة القابلية الإخصابية للتقنيات الساندة للتكاثر .

Introduction

The progestrone acts as a physiological stimulator for human sperm functions. It is present in the fertilization region with high concentration and affects on some sperm function like sperm motility, capacitation , acrosome reaction and sperm efficiency for response and interaction with zona pellucida proteins [1,2], in addition to the improvement of sperm penetration rate [3]. Previous studies revealed that the stimulation effect of follicular fluid and human granuloa media on human sperm hyperactivation and acrosome reaction, and this effect refers to the progestrone present in both above media [4]. Usually, the spermatozoa correlate and incubated in vivo in tubal fluid. After ovulation, the follicular fluid removes the correlation of spermatozoa from tubal wall and this refers to the progestrone action on spermatozoal hyperactivation [5]. Other investigators revealed to significant linear

correlation between sperm motility and percentage of sperm and showed an increase in calcium concentration as a response for progestrone, and then the positive important action of progestrone in fertilization[6,7]. The effects of progestrone depend on dosage [8], also refer to activated two types of progestrone receptors which are present on plasma membrane of human spermatozoa [9,10]. Other study determined that 0.4 mg / ml of progestrone medroxy-acetate is the optimum concentration to improve sperm quality for one hour of incubation period [11]. So this study aims to know the progestrone effects on motion parameters of astheno-spermatozoa for duration longer than one hour of incubation periods .

Materials and Methods

Semen collection and processing :-

Ten semen samples were obtained by masturbation from ten asthenospermic patients after 3 days of sexual abstinence . The specimens were allowed to liquefy at 37º C for 20 – 30 minutes. The mean data of the semen parameters were estimated, including : sperm concentration, sperm motility percent, grade activity, abnormal sperm morphology percent, leukocytes and phagocytes concentration .

The values of these parameters are considered as sperm parameters before activation.

***In vitro* sperm activation technique :-**

Two ml of each semen sample was divided into four equal splits , placed separately into four centrifuged tubes. Each 0.5 ml of semen was mixed with 1 ml of glucosaline supplemented with 20 % inactive maternal serum. The mixture was centrifuged at 2000 rpm for 5 minutes, the supernatant was discarded and the final pellet in four tubes was overlayered with glucosaline alone (control) , glucosaline with 0.1 mg / ml of progestrone medroxy-acetate, glucosaline with 0.4 mg / ml of progestrone medroxy-acetate and glucosaline with 1 mg / ml of progestrone medroxy-acetate. The four tubes were kept in an incubator at 37º C .

A drop of top part of media was aspirated after 90 and 120 minutes from each tube and examined to evaluate sperm parameters. The results were analyzed by using analysis of variance (ANOVA) and LSD to indicate the significancy[12].

Results

The results of *in vitro* sperm activation showed a significant improvement ($p < 0.001$) in all sperm parameters by using glucosaline alone or with different concentrations of progestrone medroxy-acetate compared to those values before activation in two incubation periods which were used .

The incubation of asthenosperm with glucosaline supplemented with 0.4 mg /ml of progestrone medroxy-acetate caused a significant increase ($p < 0.001$) in sperm motility percent and grade activity compared to glucosaline alone or by using 0.1 and 1 mg /ml of progestrone medroxy-acetate at 90 and 120 minutes of incubation periods (Table 1 and 2). While other values of activated sperm parameters showed insignificant differences ($p>0.05$) between glucosaline alone and different concentrations of progestrone medroxy-acetate .

Discussion

The result of the present study has shown that the incubation of asthenospermic specimens with glucosaline which supplemented with 0.4 mg /ml of progestrone medroxy-acetate caused a significant increase in sperm motility percent and grade activity compared to glucosaline alone

and other concentrations of progestrone medroxy-acetate for 90 and 120 minutes of incubation periods; this may refer to the dose-dependent effect of progestrone medroxy-acetate on sperm hyperactivation . The result agrees with [13] study , that progestrone acetate depend on dosage, and has an important role in cAMP increasing. The incubation of human spermatozoa with 100 µ mol / L of progestrone caused two peaks in cAMP increasing curve , the first one occur after half hour and the second after two hours of incubation and this increasing correlated with significant increase of hyperactivate sperm motility percent . Another study showed that using 0.4 mg / ml of progestrone medroxy – acetate in sperm activation caused a significant increase in sperm motility percent and grade activity compared to the control at 30 and 60 minutes of incubation periods [11,8] noticed that adding human follicular fluid to spermatozoa caused sperm hyperactivation for three hours , where as the sperm motility was decreased significantly after one hour without human follicular fluid .

The effects of progestrone were mediated by an increase in the formation of inositol triphosphate which increased the liberation of intracellular Ca^{+2} [14-17] and activation of adenyl cyclase which led to the increase of the generation of cAMP. Consequently cAMP increased due to activation of protein kinase A with the increased protein phosphorylation and hyperactivation of sperm motility [18-20]. So the increase of intracellular content of cAMP was correlated with significant improvement in the percentage of forward progressive spermatozoa at the fourth hour [21] and had a sign effect on the occurrence of acrosome reaction [22].

The results showed insignificant differences in recovery sperm concentration, abnormal sperm morphology percentage, white blood cells concentration with phagocytes in different treatments compared to control, this may mean that this study was performed in vitro and sperm concentration or morphology was completely determined *in vivo* .

It has been concluded, that 0.4 mg / ml of progestrone medroxy-acetate can maintain the motion parameters for 90 and 120 minutes and this duration is long enough to increase the number of sperm reaching the upper reproductive tract and may increase the fertilizing capacity in assisted reproduction .

References

1- Baldi , E. ; Luconi , M.; Bonaccorsi , L. & Forti , G. (1999) . Non-genomic effects of progestrone on spermatozoa : relevance to sperm function . Steroid Research ; 2 : 119 – 125 .

2- Baldi, E. ; Luconi , M. ; Bonaccorsi , L.; Maggi , M.; Francavilla , S.; Gabriele , A. ;Properzi , G.& Forti , G. (1999).
Non-genomic progestrone receptor on human spermatozoa : biochemical aspects and clinical implication . Steroids ; 64 : 143 – 148 .

3- Margalioth , E. J. ; Bronson , R. A. ; Cooper , G. W. & Rosenfeld , D. L. (1988). Luteal phase sera and progestrone enhance sperm penetration in the hamster egg assay . Fertility and Sterility ; 50 : 117–122 .

4- Fabri , R. ; Porcu , E. ; Lenzi , A. ; Gandini , L. ; Marsella , T. & Flamigni , C. (1998) . Follicular fluid and human granulosa cell cultures: influence on sperm kinetic parameters , hyperactivation, and acrosome reaction .Fertility and Sterility; 69: 112 – 117 .

5- Kulin , S. ; Bastians , B. A. ; Hollanders , H. M. G. ; Janssen , H. J.

M . (1994) . Human serum and follicular fluid stimulate hyperactivation of human spermatozoa after preincubation . Fertility and Sterility ; 62 : 1234-1237 .

6- Giojalas , L. (1998) . Correlation between response to progestrone and other functional parameters in human spermatozoa . Fertilty and Sterility ; 69 : 107 – 111 .

7- Ozaki , Y. ; Blomgren , K. ; ogasawara , M. S. ; Aoki ,K. ; Furuno , Y. ; Nakanishi , M. ; Sasaki , M. & Suzumori ,K. (2001) . Role of calpain in human sperm activated by progestrone for fertilization . Biol . Chem. ; 382 : 831-838 .

8- Yao , Y. ; Ro , P. & Yeung , W. S. (2000) . Effects of human follicular fluid on the capacitation and motility of human spermatozoa . Fertility and Sterility ; 73 : 680- 686 .

9- Baldi , E. ; Luconi , M. ; Bonaccorsi , L. & Forti , G. (1998) . Non-genomic effects of progestrone on spermatozoa : mechanisms of signal transduction and clinical implication . Frontiers in Bioscience ; 3 : 1051-1059 .

10- Luconi , M. ; Bonaccorsi , L. ; Maggi , M. ; Pecchioli , P. ; Krausz , C. ; Forti , G. & Baldi , E. ; (1998) .Identification and characterization of functional nongenomic progestrone receptors on human sperm membrane . Journal of Clinical Endocrinology and Metabolism ; 83 : 877- 885 .

11- Hussain , Batool Ibrahim . (2003) A study of the effect of some substances on sperm activation in vitro of asthenospermic patients . M.S.C. thesis. University of Babylon . 111 pp .

12- Scheffer , W. C. (1980) . Statistics for the biological science . 2nd edition . Scheffer , W. S. (ed) . Addison Westery publishing company . California , London . Amsterdam .

13- Parinaud , J. & Milhet , P. (1996). Progestrone induces Ca^{+2} – dependent $3^{-}:5^{-}$–cyclic adenosine monophosphate increase in human sperm . (Abst.) , J Clin Endocrinol Metab ; 81 : 1357 – 1360.

14- Machelon , V. ; Nome , F. ; Grosse , B. & Lieberherr , M. (1996) . Progestrone triggers rapid transmembrane calcium influx and / or calcium mobilization from endoplasmic reticulum , via a pertussis-insensitive G- protein in granulosa cells in relation to luteinization process . Journal of cellular Biochemistry ; 61 : 619 – 628 .

15- Puplicover , S. J. & Barratt , C. L. R. (1999) Voltage – operated Ca^{+2} channels and the acrosome reaction : which channels are present and what do they do ? . Human Reproduction ; 14 : 873 – 879 .

16- Brown , J. C. K.; Bray, C.; Stewart , P. M. ; Barratt , C. L. R. & Publicover , S. J. (2000) . Biphasic elevation of [Ca^{+2}]$_I$ in individual human spermatozoa exposed to progestrone . Developmental Biology ; 222 : 326-335 .

17- Ho , H. & Suarez , S. S. (2001) . An inositol 1, 4, 5-triphosphate receptor – gated intracellular Ca^{+2} store is involved in regulation sperm hyperactivated motility . Biology of Reproduction ; 65 : 1606 – 1615 .

18- Bonaccorsi , L. ; Krausz , C. ;Pecchioli , P. ; Forti , G. & Baldi , E. (1998) . progestrone-stimulated intracellular calcium increase in human spermatozoa is protein kinase C-independent . Molecular Human Reproduction ; 4 : 259- 268 .

19- Forti , G. ; Baldi , E. ; Krausz , C. ; Luconi , M. ; Bonaccorsi , L. ; Maggi , M. Bassi , F. & Scarselli , G.(1999) . Effect of progestrone on human spermatozoa : clinical implications . Annales d'Endocrinologie ; 60 : 107-110 .

20- Baldi , E. ; Luconi , M. ; Bonaccorsi , L. Muratori , M. & Giann , F.(2000) . Intracellular events and signaling pathways involved in sperm

acquisition of fertilizing capacity and acrosome reaction . Frontiers in Bioscience ; 1 : 110-123 .
21- Deturner , E. A. ; Aparicio , N. J. ; Turner , D. ; Schwarz stein , L. (1978) . Effect of two phosphodiestrase inhibitors , cyclic adenosine 3',5'-monophosphate , and a B- blocking agent on human sperm motility . Fertility and Sterility; 29 : 328-331 .
22- Garde , J.& Roldan , E. R. S. (2000) Stimulation of Ca^{+2}-dependent exocytosis of the sperm acrosme by cAMP acting downstream of phospholipase A2. Journal of Reproduction and Fertility; 118: 57-68 .

Table 1 Sperm function parameters before and after activation of asthenospermic patients by using glucosaline medium with different concentrations of progestrone medroxy-acetate for 90 minutes of incubation period .

Sperm parameters	Before treatment (mean ± SD)	After treatment (mean ± SD)			
		Control	Progestrone medroxy – acetate (mg / ml)		
			0.1	0.4	1
Sperm concentration ($\times 10^6$)	a 63.20 ± 22.73	b 9.60 ± 4.59	b 10.70 ± 6.32	b 11.10 ± 6.31	b 9.60 ± 5.25
Sperm motility percent %	a 35.50 ± 9.26	b 65.00 ± 6.66	b 62.00 ± 5.86	c 73.50 ± 8.18	b 63.50 ± 11.31
Grade activity	a 2.18 ± 0.44	b 3.54 ± 0.17	b 3.47 ± 0.17	c 3.76 ± 0.25	b 3.50 ± 0.29
Abnormal sperm morphology	a 44.84 ± 13.66	b 17.24 ± 6.66	b 18.18 ± 4.88	b 18.80 ± 7.12	b 20.34 ± 5.78
Leukocytes and phagocytes concentration ($\times 10^6$)	a 2.10 ± 1.72	b 0.10 ± 0.31	b 0.10 ± 0.31	b 0.15 ± 0.33	b 0.17 ± 0.33

Patient number = 10
$p < 0.001$ significant difference .
Different letters indicate for significancy .

Table 2 Sperm function parameters before and after activation of asthenospermic patients by using glucosaline medium with different concentrations of progestrone medroxy-acetate for 120 minutes of incubation period .

Sperm parameters	Before treatment (mean ± SD)	After treatment (mean ± SD)			
		Control	Progestrone medroxy – acetate (mg / ml)		
			0.1	0.4	1
Sperm concentration ($\times 10^6$)	a 63.20 ± 22.73	b 9.60 ± 4.59	b 11.30 ± 6.32	b 11.60 ± 6.31	b 10.10 ± 5.25
Sperm motility percent %	a 35.50 ± 9.26	b 64.50 ± 5.50	b 62.00 ± 7.52	c 73.50 ± 7.47	b 61.50 ± 11.06
Grade activity	a 2.18 ± 0.44	b 3.49 ± 0.15	b 3.41 ± 0.19	c 3.71 ± 0.24	b 3.39 ± 0.95
Abnormal sperm morphology	a 44.84 ± 13.66	b 18.89 ± 6.88	b 19.80 ± 7.99	b 17.20 ± 7.43	b 18.69 ± 6.54
Leukocytes and phagocytes concentration ($\times 10^6$)	a 2.10 ± 1.72	b 0.12 ± 0.31	b 0.17 ± 0.33	b 0.15 ± 0.34	b 0.12 ± 0.33

Patient number = 10
$p < 0.001$ significant difference .
Different letters indicate significancy .