

oviductal flushing medium support *in vitro* fertilization of mice :A biochemical and hormonal study⁺

Saad S. Al-Dujaily^{*}

Munaf S. Daoud^{**}

Abstract

The objective of the present study is to measure some biochemical and hormonal contents of oviductal flushing medium (OFM) used for *in vitro* fertilization and embryonic development of mice as a model for mammals.

Oviductal flushing medium of superovulated and spontaneously ovulated mice was analyzed for certain biochemical and hormonal contents. Amino acids (taurine, alanine, aspartate, glutamine and glycine) were measured using HPLC. Glucose and fructose were estimated using glucose oxidase method and fructose indol-reagent colorimetric method, respectively. Magnesium, calcium, sodium and potassium ions were determined using flame photometry and atomic absorption spectrophotometry. Luteinizing hormone (LH) and follicular stimulating hormone (FSH) were measured by enzyme linked fluorescent assay technique.

The results of this investigation show that there is no significant ($P > 0.05$) difference in the concentration of taurine, glutamine and alanine in the OFM of SUO and SO mice. The OFM contents of aspartate and glycine in SUO is significantly ($P < 0.05$) higher than that of SO group. A significant ($P < 0.05$) decrease was observed in the concentration of glucose ($\mu\text{mol/L}$) in OFM of SUO compared to SO females. The concentration of Ca^{++} in the OFM of SUO ($66.5 \mu\text{g/ml}$) was significantly ($P < 0.05$) higher than that of SO mice ($60.2 \mu\text{g/ml}$). There was a significant ($P < 0.05$) decrease in the level of LH in the OFM of SUO compared to SO mice. It is concluded from the present study that the OFM of superovulated mice contains higher amounts of different amino acids, electrolytes and hormones that lead to offer a proper environment for *in vitro* fertilization and embryonic development of early cleavage stages in mice.

المستخلص

هدفت الدراسة الحالية لتحديد كمية بعض محتويات الكيمياء الحيوية والهرمونية للسائل الدافق لقناة البيض والمستخدم في الإخصاب ونمو الأجنة في الزجاجة للفئران والتي استخدمت نموذجاً للبيان. حلل الوسط الدافق لقناة البيض من فئران محفزة وأخرى غير محفزة بالهرمونات تحليلاً كيميائياً وهرمونياً فقد تم حساب كمية بعض الأحماض الأمينية (taurine, alanine, aspartate, glutamine, glycine) باستخدام HPLC. تم تقدير الجلوكوز والفركتوز باستخدام طريقة أكسدة الجلوكوز وطريقة صبغ الإندول للفركتوز، على التوالي. تم تحديد المغنيسيوم، الكالسيوم، الصوديوم والبوتاسيوم باستخدام طيف الفلوميترية اللهب وطيف الامتصاص الذري. تم قياس الهرمون اللوتيني (LH) والهرمون المنبهر للبيضة (FSH) باستخدام طريقة الاختبار الفلوريمنس المرتبطة بالإنزيم.

⁺Received on 9/1/2006

Accepted on : 4/12/2006

^{*}Assistant Professor, Chairman, Department of Clinical Reproductive Physiology, IVF Institute, Al-Nahrain University

^{**}. Assistant Professor, College of Medicine, Baghdad University, Baghdad -Iraq.

glutamine and glycine) باستخدام جهاز HPLC .تم تقدير كمية سكر الكلوكوز والفركتوز باستخدام طريقة glucose oxidase وطريقة fructose indol-reagent colormetric على التوالي. حددت مستويات أيونات المنغنيز والكالسيوم والصوديوم والبوتاسيوم باستخدام طريقتي flame photometry and atomic absorption spectrophotometry . تم حساب هرموني المحفز الجريبي (FSH) واللوتيني (LH) باستخدام تقنية Enzyme linked fluorescent assay. أظهرت النتائج الحالية عدم وجود فرق معنوي ($P>0.05$) في تركيز التورين والكلوتامين والألنين في محتوى السائل الدافق من قناة البيض بين مجموعة الفئران المحفزة بالهرمونات وغير المحفزة. محتوى السائل الدافق من الأسبريت والكلايسين في قناة بيض الفئران المحفزة بالهرمونات أعلى معنوياً ($P<0.05$) من الفئران غير المحفزة . وجد انخفاض معنوي ($P<0.05$) في تركيز الكلوكوز في السائل الدافق لقناة بيض الفئران المحفزة بالهرمونات بما يقابلها في الفئران غير المحفزة. تركيز الكالسيوم في الفئران المحفزة بالهرمونات (٦٦,٥ ملغم/ملتر) أعلى معنوياً ($P<0.05$) من تركيزه في الفئران غير المحفزة بالهرمونات (٦٠,٢ ملغم/ملتر) . كما لوحظ وجود انخفاض معنوي ($P<0.05$) في معدل الهرمون اللوتيني الموجود في السائل الدافق لفئران محفزة بالهرمونات بالمقارنة مع فئران غير محفزة. لذا نستنتج من هذه الدراسة أن السائل الدافق من قناة بيض الفئران المحفزة بالهرمونات يحتوي على كميات أكبر من مختلف الأحماض الأمينية والأيونات والهرمونات مما أدى الى توفير بيئة ملائمة للأخصاب في الزجاج والتطور الجنيني لمراحل الأقسام المبكرة في الفئران.

Introduction

Fluid present in the lumen of the oviduct is formed by transudation from the blood and active secretion from the oviductal mucosa and varies in composition, rheological properties and volume depending on the stage of sex cycle[1]. It has been found that the constituents of accumulated oviductal fluid helps in maintaining sperm survival and hypermotility *in vivo* ,[2]. *In vitro*, different media were added to support fertilization and embryonic development processes .It has been reported that the medium used must contain amino acids, vitamins, insulin epidermal growth factor ,and minerals [3]. Mice OFM improves the activation of human and mice sperm functions [4]. At the same time ,this medium was shown to enhance the fertilization rate and embryonic development in different early cleavage stages [5]. However, the constituent of this medium is still obscure . Therefore ,the aim of the study is to measure and analyze some certain biochemical and hormonal constituents that are found at oviductal fluid of superovulated (SOU) and spontaneously ovulated(SO) mice to answer the positive correlation between OFM and increase in fertilization rate(FR) and embryonic development(ED) following in vitro fertilization(IVF) of mice ova .

Materials and Methods

Healthy mature Balb/c female mice of 6-8 weeks old were used in this study. Superovulation were done by 7.5 I.U. of pregnant mare's serum gonadotropin(Folligon®, Intervet international B.V., Holland) followed by 48hours 7.5 I.U. of human Chorionic gonadotropin(Pregnyl®, Organon Oss, Holland) both

injected intra-peritoneally.

Oviductal flushing median were collected from superovulated (SUO) and spontaneously ovulated (SO) female mice as described by Al-Dujaily, et al.[5].The final volume from flushing oviductal tubes with cumulus cells was one ml(0.5 ml from each tube).

-Biochemical analysis of OFM were performed on:

- 1- **Amino acids(AAs)**; Taurine , alanine ,aspartate , glutamine and glycine are estimated by high performance liquid chromatography(HPLC) technique[6]. These AAs were analyzed by Shimadzu LC-10AVT Liquid chromatograph equipped with UV-visible detector model 10SPD. The eluted peaks were drawn by LC-6A data processors. All were obtained from Shimadzu(Koyoto, Japan).
- 2- **Monosaccharides** ; Glucose concentration was estimated using the glucose oxidase method[7].The kit was obtained from Cromatest ,Linear Chemicals SL. Barcelona, Spain. Fructose concentration was determined by the colorimetric method using indol reagent[8].
- 3- **Electrolytes**; as sodium and potassium ions were measured using Flamephotometer [9] . Calcium and Magnesium ions estimated by atomic absorption spectrophotometer 679(Shimadzu, Koyoto-Japan)[10,11].
- 4-**Gonadotropic hormones** ;The gonadotropic hormones namely; luteinizing hormone (LH) and follicular stimulating hormone (FSH) were estimated using enzyme linked fluorescent assay(ELFA) by Mini- Vidas[®] ,(Biomerieux Company ,France)[12].

Statistical Analysis

Comparison of different biochemical and hormonal constituents in OFM of SUO and SO mice was performed using Student's t-test .The P value of less than 0.05 was considered significant [13].

Results

The results of amino acids and monosaccharide contents in OFM of SUO and SO mice are shown in Table -1. There is no significant ($P>0.05$) difference in the concentration of taurine ,glutamine and alanine between SUO compared to SO mice. The OFM contents of aspartate and glycine in SUO is significantly($P<0.05$) higher than that of SO group(87 and 32.85 $\mu\text{g/ml}$ vs. 74 and 28.57 $\mu\text{g/ml}$,respectively).A significant ($P<0.05$) decrease is observed in the concentration of glucose ($\mu\text{mol/L}$) in OFM of SUO compared to SO females.

In Table-2a ,the concentration of Ca^{++} in the OFM of SUO (66.5 $\mu\text{g/ml}$) is significantly ($P<0.05$) higher than that of SO mice(60.2 $\mu\text{g/ml}$).There is an increase in the concentrations of other electrolytes namely; Na^+ , K^+ Mg^{++} in the flushed medium of SUO compared to SO but this difference does not reach the significant level.Table-2b shows ,the levels of FSH and LH hormones in OFM of SUO and SO mice. There is a significant($P<0.05$) decrease in the level of LH in the OFM of SUO mice compared to SO (2.8 vs. 6.3 $\mu\text{I.U/ml}$).

Table-1:Amino acids and monosaccharide concentrations in theOFM of SUO and SO mice

	concentration ($\mu\text{g/ml}$)
--	------------------------------------

Amino acids	SUO	SO
Taurine	234.20±0.30	234±0.64
Alanine	274.6±1.26	281.4±1.48
Aspartate	87.0±2.20*	74.0±1.07
Glutamine	128.0±1.70	128.0±1.20
Glycine	32.85±1.30*	28.57±0.67
Monosaccharide	concentration ((μmol/L)	
	SUO	SO
Glucose	5.5±1.3*	8.3±1.2
Fructose	0.16±0.13	0.22±0.08

Table-2: Concentrations of electrolytes and Hormones in the OFMof SUO and SO mice

a-Electrolytes	concentration (μg/ml)	
	SUO	SO
Na ⁺	119.2±3.8	117.8±7.6
K ⁺	78.8±4.1	72.6±3.8
Ca ⁺⁺	66.5±2.20*	60.2±1.37
Mg ⁺⁺	8.92±0.82	7.62±0.64
b-Hormones	concentration ((μI.U/ml)	
	SUO	SO
LH	2.8±0.8	6.3±1.2*
FSH	1.5±0.6	1.1±0.2

The values are mean ± SEM

*P<0.05; significantly different from SO mice

SUO: superovulated mice

SO: spontaneously ovulated mice

Discussion

In this study ,it is well established that amino acids can improve preimplantation development of mice embryos *in vitro*[14].Alanine, glutamine, glycine and taurine are abundant in the oviductal lumen. These major amino acids were detected in the OFM of SUO and SO mice . Our findings are compatible with observations made by Dumoulin et al [15]and Chatote et al.[16,17] in the presence of taurine and glutamine with other amino acids[18] in the oviductal fluid and its positive importance on preimplantation development of mice embryos and their support of human and bovine blastocyst formation *in vitro*[18,19].Moreover, glycine play important role in protection mouse conceptuses from detrimental effect of the inorganic ions in oviductal fluid on the development [20].In our finding glycine concentration is higher in the OFM of SUO than SO mice that may lead to increase the FR and ED of mice in our previous study[5].

The glucose concentration is lower in the OFM of SUO than that of SO , an observation that is inconsistent with other studies which found that oviductal pyruvate concentration is significantly higher and glucose concentration is significantly lower in the presence of cumulus cells,[21,22,23].It has also been observed by Chatot, et al.[16] that glucose may play a detrimental effect on embryonic development at early cleavage stages.Thus the lower concentration of glucose in the OFM of SUO may enhance preimplantation embryonic process than

OFM of SO.

The significant increase in calcium ion in OFM of SUO compared to SO can be explained on the observation made by Baker, et al. [24]. Ca^{++} ion was found to be a powerful ,potential regulator of tyrosine phosphorylation during sperm capacitation in both human and mouse spermatozoa .

The study reveals a decrease in LH hormone in the OFM of SUO mice. This finding may play a fundamental factor in order to have an intact oocyte quality and then to have normal fertilization process. It is becoming increasingly clear that LH has a central role in the process of maturation of the oocyte. The hypersecretion or inappropriate secretion of LH impairs oocyte quality and may cause infertility and miscarriage independently of any impairment of ovulation[25].

Conclusion

It is concluded from this study that OFM of SUO mice can provide a suitable environment for fertilization process and embryonic development *in vitro*.

References

- 1- Croxatto H. and Villalon M .Oocyte transport.In:*Biology and Pathology of the Oocyte*,Trounson A and Goden R(eds), Cambridge Press,UK;pp:253-276, 2003.
- 2- Lapointe S., Bilodeau J., Lemieux D., Asselin E., Fortier M., and Sirard M. Epithelial and stromal uterine cells cultured *in vitro* protect bovine sperm from hydrogen peroxide. *Theriogenology*;Vol. 54(3): 355-69. 2000
- 3- Gardner D.K and Sakkas D. "Mouse embryo cleavage, metabolism and viability: role of medium composition". *Human Reproduction*;8(2):288-295. 1993
- 4- Al-Dujaily S, Albarazanchi M, and Fakhridin M. "Use of mice oviductal flushing medium *in vitro* to activate the human and mice sperm functions".*J Babylon University*;Vol.4:508-514, 1999.
- 5- Al-Dujaily S,Al-Mashhadani A Al-Ghazi M. and Al-Malki R ."Oviductal flushing medium support *in vitro* fertilization of mice: experimental model for mammals". *Ann. College of Medicine Mosul*; "Vol,30(1):6-10, 2004.
- 6- Hancock W. and Sparrow J."HPLC analysis of biological compounds:A laboratory guide".*Chromatographic Science Sserial* ;Vol.26,New York, Marcel Dekker,Inc.,1984.
- 7- Trindr P. "Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor ".*Ann Clin Biochem*;Vol.6:24-27, 1969
- 8- Karvonen M .J.,and Malm M. Colorimetric determination of fructose with indol.Scan.*J Cl Lab Invest.*;Vol.7:305-307. 1955
- 9- Varley H.Gowenlock A. and Bell M.*Practical Clinical Biochemistry* .Vol.15th ed,William Heinemann Books Ltd.,London,pp:180-200.1980.
- 10-Bowers G and Pybus J."Total calcium in serum by atomic absorption spectrophotometry". In:*Standard Methods Of Clinical Chemistry*.Vol,7,S Meites ,ed.,New York, Academic Press ,pp:143,1972.
- 11- Tietz N.*Fundemental Of Clinical Chemistry*,3rd Ed,W.B.Saunders Co., Philadelphia ,pp:521-524,1987.

- 12- Al-Kaabi M.A. *Changes Associated With Ovulation Induction In Infertile Women*. High Diploma Thesis, Institute of Embryo Research and Infertility Treatment, Baghdad University, pp:48-57, 2005
- 13- Gill J. *Design and Analysis Of Experiments In The Animals and Science*. 3rd(ed.), Iowa .The Iowa state University Press,;pp:1-133, 1978
- 14- Van winkle L , and Dickinson H. "Differences in amino acid content of preimplantation mouse embryos that develop *in vitro* versus *in vivo*: *in vitro* effects of five amino acids that are abundant in oviductal secretions" .*Biol Reprod*; Vol. 52(1):96-104. 1995
- 15- Dumoulin J. Evers J, Bars M. Pieters H and Gerardts ,J. "Positive effect of taurine on preimplantation development of mouse embryo *in vitro*".*J Reprod Fert*.Vol. 1;94:373-380, 1992
- 16- Chatot C, Ziomek C, Baveister B, Lewis J, and Torresl A.S. : "Improved culture medium supports development of random-bred 1-cell mouse embryos *in vitro*". *J. Reprod Fertil*; Vol. 86: 679-688, 1989
- 17- Devrekor F.,Bergh J.,Biramane M.,Winston K.M.and Hardy K. "Effects of taurine on human embryo development *in vitro*". *Human Reproduction*;Vol.14(9): 2350-2356 . 1999
- 18- Devrekor F.,Hardy M.,BerghV.,NvanninA.,Emiliani S. and Englert.Y." Amino acids promote human blastocysts development *in vitro*".*Hum. Reprod.*;Vol.16(4),749-756. 2001
- 19- Gopichandran N., and Leese H.J. "Metabolic characterization of the bovine blastocyst, inner cell mass, trophectoderm and blastocoel fluid". *Reproduction*.;Vol. 126(3):299-308,2003.
- 20- Van winkle L., Haghighat N., and Campione A.L. "Glycine protects preimplantation mouse conceptuses from a detrimental effect on development of the inorganic ions in oviductal fluid" . *J. Exp. Zool.*;Vol. 253 (2) : 215 -219 ,1990.
- 21- Hammer M.A.,and Baltz J.M.."Beta-alanine but not taurine can function as an organic osmolyte in preimplantation mouse embryos cultured from fertilized eggs". *Mol Reprod Dev* . ;Vol. 66 (2) : 153 – 161, 2003.
- 22- Leese H.J.and Barton A.M. "Production of pyruvate by isolated mouse cumulus cells".*J Exp Zool* . ;Vol.234(2):231-236. 1985
- 23- Harris S.E., Gopichandran N., Picton H.M., Leese H.J.,and Orsi N.M ."Nutrient concentrations in murine follicular fluid and the female reproductive tract" . *Theriogenology*.;Vol.64(4):992-1006. 2005
- 24- Baker M.A.,Hetherington L.,Ecroyd H.,Roman S.D.and Aitken R.J."Analysis of the mechanism by which calcium negatively regulates the tyrosine phosphorylation cascade associated with the sperm capacitation ". *J Cell Science* ;Vol. 117: 211- 222, 2004.
- 25- Homburg R. and Shelef M. Factors affecting oocyte quality. In: *Biology and Pathology Of The Oocyte*, Trounson A and Goden R. (eds), Cambridge Press,UK;pp: 277-291, 2003.