Pregnancy Rate Following In Vitro Sperm Activation and IntraUterine Insemination.

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Abstract:
In vitro sperm activation for 30 samples of asthenospermic patients were performed in Embryo Research and Infertility Treatment Center /Baghdad University. Sperm activation was induced in Earl's and Ham's F-10 culture media, supplemented with 30%inactive maternal serum using centrifugation/wash-out technique. Significant improvement ($p<0.001$) in sperm motility was obtained following activation compared to those before activation. Earl's and Ham's F-10 media using centrifugation/wash-out technique can be used confidently for the selection of active motile sperm with lowest percentage abnormality, and infectious product free for the purpose of Assistant Reproductive Technology (ART). The pregnancy rate per cycle following IntraUterine Insemination (IUI) with hyperactivated sperm in Earl's media was (30%) while with Ham's F-10 was (28.3%). The use of Earl's or Ham's F-10 media seems to be identical on sperm transfer media for (IUI) purpose

الخلاصة
تم تنشيط ٣٠ عينة مني بشرية في الزجاج لمرضى العقم الذين يعانون من وهن النطف راجعوا مركز أبحاث الأجنة وعلاج العقم/جامعة بغداد. تم تنشيط نطف مرضى العقم باستخدام الوسطان الزرعيان ايرل وهام أف -١٠
المجهز ب ٣٠% من مصل الدم غير الفعال و باستخدام تقنية الغسل والنبذ .لوحظ تحسنا معنويا ($p<0.001$) في حركة النطف بعد التنشيط مقارنة بنسبها قبل التنشيط.على ضوء النتائج يمكن استخدام الأوساط ايرل و هام أف-١٠ وتقنية الغسل والنبذ لعزل النطف المتحركة بنشاط، وبنسب قليلة من النطف اللاسوية وأيضا معدلات قليلة أو معدومة من الخلايا الالتهابية لغرض استخدامها في التقنيات المساعدة على الإنجاب .أن معدل الحمل بعد اجراء عمليات التلقيح في جوف الرحم باستعمال النطف المنشطة باستخدام الوسط ايرل بلغ (٣٠%)،بينما باستخدام الوسط هام أ ف-١٠ بلغت (٢٨,٣%) لكل دورة شهرية. يستنتج أن الوسطين ايرل و هام أف-١٠ممكن استخدامهم كوسط لتنشيط النطف ونقلها الى التجويف الرحمي.

Introduction

The necessity of high quality sperm preparation may appear less important as (ART) advances. However the study of intracytoplasmic sperm injection (ICSI) in human and non-human primates has revealed that the use of randomly selected sperm for this procedure result in various anomalies of sperm decondensation and embryonic development (1).Thus semen preparation for new ART is an important step in the treatment of infertile couples. Native semen contain substance which impair sperm motility , acorsome reaction and fertilizing ability of the sperm (2).Intrauterine insemination is considered to be least invasive and first line therapy for couples presented with subfertility(3).IUI increases the number of sperm into the fallopian tubes where fertilization take place(4). Previous studies showed that IUI of washed motile spermatozoa from male with asthenospermia was reported to enhance conception rate (5.6).This study demonstrate the signifant use of hyperactivated sperm to improve pregnancy rate following IUI.

Materials and Methods

Patients:

In this study thirty infertile couples were involved during their attendance
at Embryo Research and Infertility Treatment Center /Baghdad University The infertile male patients were with mean age of 36 years, the clinical data of those patients are shown in table (1).The following minimal investigations were carried out for the enrolled couples: complete physical examination, both systemic and local. All the female underwent ultra sonography (u/s) of the pelvis, Hysterosalpingiography for bubal patency, serum prolactin and serum progesterone cycle day 21 was carried out.

Ovarian Stimulation Program:
Ovarian stimulation was induced by a daily dose of 100mg clomiphene citrate (cc , clomid,merrell, company, England) , from day two of menstrual cycle until day 6.
Follicular development was monitored by ultrasound. When one or more dominant follicles measured 18mm in diameter by u/s on cycle day 12 , human chorionic gonadotropins (HCG, pregnyl, organon, Holland) was given intramuscularly in a dose of 5000 IU to 10000 IU , to induce ovulation. After 24-36 hours insemination was performed.

Serum Inactivation:
Serum was withdrawn from female undergoing ovarian stimulation program at cycle day 13.The serum was heat inactivated in water both at 56°c for 30 min and stored at -20°c until use.

Semen collection and in vitro sperm activation:
Semen specimens were collected after 2-3 days of abstinence by masturbation. The specimens were incubated in 37 °C for 30 minutes to liquefy. Seminal fluid analysis was performed to determine the sperm concentration, sperm motility percent, grade motility, abnormal sperm morphology percent and concentration of leukocytes and phagocytes. Sperm activation was done using the centrifugation/wash-out technique using the following culture media:-

1. Earl's culture medium supplemented with 30% serum concentration.
2. Ham's F-10 media supplemented with 30% serum concentration.

One ml of aliquot of the semen samples was diluted with 3ml of the above media, centrifuged at 2000(rpm) for 5 minutes. The supernatant was discarded, and the pellet was gently covered with 1ml of the media and incubated for 30 minutes at 37°C. A drop from the upper 1/3 of the supernatant was taken and examined microscopically. The effect of the prepared media on sperm parameters was studied. These parameters were recovered sperm concentration, sperm motility percent, grading of forward sperm progression and percent normal morphology. The efficiency of the culture media to get rid of the infectious products was also studied.

5**. Intrauterine Insemination:**
The recovered sperm by in vitro sperm activation were aspirated by a gauge-4 intravenous catheter (portex, limited, Kent, UK) which was connected to 2ml syringe. The female was in lithotomy position; the cervix was exposed by a bivalve speculum and cleaned with sterile culture medium. The tip of the catheter was gently passed through the cervical canal and the internal os into the uterine cavity, after reaching the fundus , the catheter was drawn few mm. The content of the catheter was injected slowly and about 0.5-1 ml of the media with the recovered sperm (5-25 million/ml)was injected inside the uterus. After 15-30 minutes of resting in spine position the patient resumed normal activity. The quantitative estimation of Beta-human chorionic gonadotropins (B-HCG) and progesterone were performed on some patient's blood fourteen days following insemination to check pregnancy test. Clinical pregnancy symptoms and positive B-HCG test with us documentation of gestational sac were reported. A chemical pregnancy was associated with transiently

elevated B-HCG concentration without fetal or trophoblastic tissues presented in u/s.
Once pregnancy has been diagnosed, the patient was referred to gynecologist to follow up the course of her pregnancy.

Statistical Analysis Of The data:
Statistical analysis accomplished by using analysis of variance, the level of significance was assessed by student t-test and chi square test.(7) .

Results

The results of sperm parameters {concentration, motility, grade activity, percent abnormal morphology and leukocyte and phagocytes concentration} were analyzed before and after hyperactivation with both culture media.
Sperm motility ,grade activity were significantly improved ($p < 0.001$) after incubation with 30% inactive maternal serum concentration and Earl's culture medium .Abnormal sperm morphology percent and leukocytes and phagocytes concentration significantly lowered ($p < 0.001$) following hyperactivation as in table(2). Table (3) shows the effect of 30% serum concentration with Ham's F-10 medium on sperm activation invitro . Sperm motility percent, grade activity, abnormal sperm morphology percent and leukocyte concentration were significantly improved ($p < 0.001$) after hyperactivation.
Figure (1) shows that 30% serum concentration gave similar results regarding percent
sperm motility with both culture media.
The results of IUI with sperm activation in vitro using centrifugation technique are shown in table (4).The effect of Earl's and Ham'sF-10 media on pregnancy rate per cycle was studied. A total of 15 couples underwent ovarian stimulation program and IUI with hyperactivated husband's sperms .The over all pregnancy rate per cycle was found to be 29.4% the use of Earl's and Ham's F-10 media with maternal inactivated midovulatory serum have insignificant difference as transfer media,(30% versus 28.3%) as shown in table (4) .

Discussion

Most men seen for infertility have reduced sperm concentration, motility, and morphology, often in combination .In this study 30 asthenospermic infertile men were involved . Sperm function tests were analyzed before and after hyperactivation invitro using centrifugation technique. There were significant reduction in sperm concentration when using Earl's and Ham's f-10 media with 30% inactive maternal serum. These findings are in agreement with that of Karlstorm etal.(8), although the technique was different . This decrease in sperm concentration is due to elimination of dead , non-motile and abnormal spermatozoa from native semen by the centrifugation/ wash-out technique .Al-Hady etal. Showed same significant improvement in sperm parameters, but he used caffeine instead of serum (9). The results of activation of asthenospermic semen samples upon using Earl's and Ham's F-10 media with 30% serum concentrations are in accordance with that of Ho, etal. (10),even better than the results of Oehninger (11) they used semen samples of sperm motility less than 22.6% while those involved in our study was 36.68% . The media contain inorganic ions (Sodium, Calcium, Potassium, Magnesium, and Phosphate) and pyruvate as source of energy. These substances may be responsible for sperm activation as well as the induction of sperm capacitation. The presence of steroids and gonadotropin hormones in serum of the stimulated females which are used for sperm activation in vitro in this study maybe responsible for improved sperm motility. The addition of pasteurized plasma protein solution to the culture media provides safe and optimal condition for sperm activation and culturing embryo for ART (12), and found to have the same effect when added instead of inactive serum.
The percentage of normal forms of spermatozoa was signifecantly higher

($p<0.001$) after invitro sperm activation ,this may be due to the effect of the culture media with centrifugation /wash-out technique in elimination of abnormal forms of the sperms, similar observation was also reported by Andelz (13),and francavilla (14) .The higher percent normal sperm morphology obtained in our study may be due to semen samples selection which were predominantly of asthenospermic nature rather than teratospermic nature , and the centrifugation itself may also be help to eliminate abnormal sperm forms .Several reports have documented an association between high level of white blood cells (WBC) in semen and male infertility (15). Experiment data indicate that WBC products can have determental effect on fertilizing ability of human spermatozoa in the zone free hamster ova penetration assay (16), and the soluble products of activated lymphocytes and granulocytes can inhibit sperm motility invitro (17). Removal of the lymphokines and/or cytokines and reduction in the form of free oxygen radicals after sperm preparation. This may result in a better sperm fertilizing abilities in vitro and in vivo (18).In our study the centrifugation technique is used for sperm activation significantly reduced leukocytes and phagocytes contamination of sperm that migrate to the top layer after incubation. The prevention of sperm contamination may be due to the supplementation of culture media with antibiotics (ampicillin).Our patients were treated with proper antibiotics before sperm activation was performed. Earl's and Ham's F-10 salts have nearly the same ions concentration except for calcium ions which are higher in Earl's medium (264 mg/L versus 33.3 mg/L) (19).This makes Earl's and Ham's F-10 media suitable for sperm activation in vitro the purpose of ART.

The practice of IUI with treated motile and morphologically normal spermatozoa, involves by passing the cervical mucus barrier resulting an increase gametes at the site of fertilization .Success rate in IUI is undoubtedly the result of better preparation procedure enhancing the quality of sperm sample (20). The combination of induction of ovulation. In vitro sperms activation and IUI will increase the pregnancy by 4% to 9% per cycle. This may be due to increase the number of oocytes released , better timing of insemination or correcting subtle ovulation defect(10). The results of Sperm IntraUterine Transfer (SIUT) of the present study support this use of hyper activated sperm of asthenospermic semen for treatment of male factor infertility. Five pregnancies resulted among 15 infertile couples under went 17 treatment cycles with average 1.1 cycle/couple. The pregnancy rate/cycle index was 29.4% (5/17) for all couples treated achieved pregnancy. The result of sperm processing and IUI in male factor patients have been quite variable. Belker and Cook (21) reported pregnancy of 28% per cycle among men with asthnospermia alone or in combination with aligospermia. Zafer etal.(22) showed pregnancy rate of 13.3% this may be due to involvement of different causes of infertility rather than the asthenospermic group involved in our study. In conclusion proper centrifugation technique and IUI may be beneficial option for treating asthenospermic infertile couples.

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Table(1) Clinical data on infertile male patients.

Patient clinical data	Number of patients	Percentage %
Patients with primary infertility	27\30	90
Patients with secondary infertility	3\30	10
Patients with seminal fluid infection	16\30	60

Table (2) The effect of sperm activation in vitro on sperm function using 30% inactive maternal serum with Earl's culture medium, using centrifugation technique (2000 rpm) for 5 minutes, following 30 minutes incubation period
(Values are mean ± SE)

Sperm different from corresponding values function test	Before activation*	After activation*
Concentration (million/ml)	47.82±4.57	8.47±1.58**
Sperm motility Percent	42.00±3.04	85.00±5.86**
Grade motility***	1.76±0.09	2.87±0.19**
Abnormal sperm morphology Percent	45.33±3.95	10.67±6.72**
Leukocytes and phagocytes concentration (million/ml)	4.26±0.61	0.13±0.13**

* number of patient per group=15
** $p<0.001$; significantly different from corresponding values.
*** 1.poor sperm progression
2. fair sperm progression
3. good sperm progression
4.very good sperm progression
5.excellent sperm progression

Table(3) The effect of sperm activation invitro on sperm function using 30% inactive maternal serum with Ham's F-10 medium, using centrifugation technique (2000 rpm) for 5 minutes, following 30 minutes incubation period
(values are mean ± SE)

Sperm function test	Before activation*	After activation*
Concentration (million/ml)	39.33±4.45	6.80±1.45**
Sperm motility percent	30.87±2.15	75.33±5.49**
Grade motility	1.57±0.05	2.67±0.14**
Abnormal sperm morphology percent	44.67±0.33	1.33±0.91**
Leukocytes and phagocytes concentration(million/ml)	4.20±0.87	None **

*number of patient per group=15
** $p<0.001$; significantly different from corresponding values

Table (4) The effect of sperm transfer media on pregnancy rate in infertile patients , following sperm intrauterine transfer.(SIUT).

Culture media	Number of cycle pregnant (percent)	Cycle number
Earl's	3 (30%)	10
Ham's	2 (28.3%)	7
Total	5 (29.4%)	17

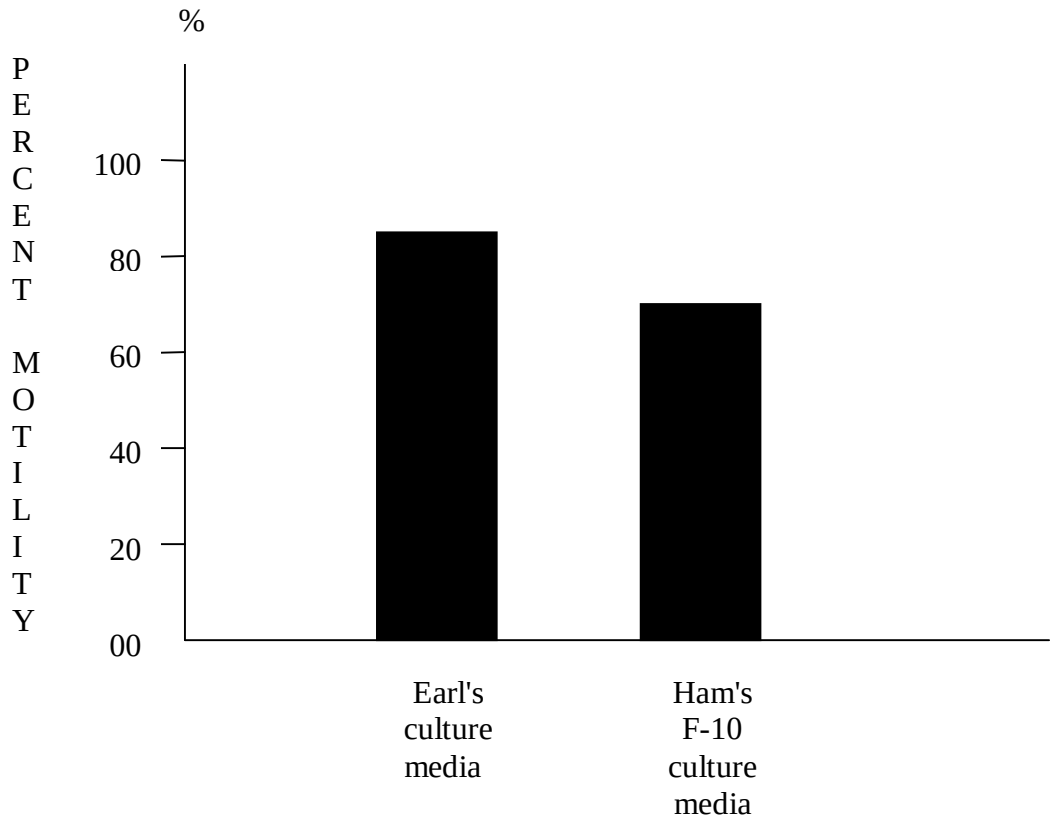

Figure 1 The effect of culture media (Earl's and Ham's F-10) with 30% inactive maternal serum on sperm motility in vitro following 30 minutes incubation period with centrifugation/wash-out technique (mean). P.>0.05; not significant . number of patient , per group=15.