The Effect of Maternal Serum Concentrations on in vitro Sperm Activation *and* Pregnancy Rate in Infertile Patients

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Summary:

Background: *Infertility is a worldwide problem. It affects 10-15% of couples, but its pattern varies from one part of the world to another. Infertility affect about 28% of the Iraqi population, and primary and secondary infertility account for 80% and 20% of cases respectively.*
Objective: *the objective of this work is to study the effected of various concentrations of maternal serum using simple layer and wash with the swim- up techniques to improve sperm activity.*
Subject & methods: *In vitro sperm activation was performed in 310 infertile patients by using modified Earl's Medium supplemented with either 20% or 30% inactive maternal serum. Simple layer and centrifugation-wash out techniques were applied for hyper-activation of asthenozoospermic infertile semen. The effect of serum concentration and sperm activation techniques on pregnancy rate were studied.*
Results: *The sperm concentration and motility were significantly improved following sperm activation in vitro by using 20% and 30% maternal serum concentrations and simple layer technique compared with centrifugation-wash and swim up technique. Superovulation and intrauterine insemination (SO-IUI) were performed with hyperactive sperm in 68 cycles. The pregnancy rate per cycle in the 20% serum concentration group was 40.60% and in the 30% serum group was 44.40% (P>0.05)*
Conclusion: *. The results of the present study indicate the prognostic potential and clinical significance of sperm activation in vitro and SO-IUI in infertile patients.*
Keywords*: Infertility, maternal serum, sperm activation, and pregnancy rate*

Introduction:

Infertility affects about 28% of the Iraqi population, and primary and secondary infertility account for 80% and 20% of the cases respectively (1). There are two major centers involved in the treatment of infertility in Iraq. The first IVF center is Kamal Al-Samaraei IVF Center and the second is Baghdad IVF Center, which is located in Baghdad Teaching Hospital. Most male infertility in Iraq is obstructive or non-obstructive azoospermia, oligospermia, astherospermia, teratospermic or a combination of these anomalies(2).

Infertility is a worldwide problem. It affects 10-15% of the couples (3), but its pattern varies from one part of the world to another (4). Recently it has been shown that infertility might affect 15-25% of the couples in United States (5). The male partner of the infertile couple represents about half of the cases (5,6). Abnormality of sperm motility and morphology is the most common cause of male infertility. Sperms have to travel a long distance to reach the site of fertilization in the fallopian tube and decreased motility may cause infertility (7,8). Various techniques are used to deliver spermatozoa to improve the probability of conception in couples with male infertility (9).In the last few decades, several techniques have been reported as successful including ovarian stimulation, intrauterine insemination, and *in vitro* fertilization (10,11). No data in the literature are available on the use of human inactive serum concentration on activation of low motility sperm and intrauterine insemination.

The objectives of this study were to study the effects of various concentrations of maternal serum using simple layer and wash method and compared with the swim-up techniques to improve the sperm activity.

MATERIAL AND METHODS:

Seminal analysis was performed for total of 465 subjects (155 fertile and 310 infertile patients). Age of patients, body weight, and blood groups were recorded.

Ovarian Stimulation: Ovarian stimulation was induced by clomiphene citrate (clomid) 50mg twice daily started from day 2 to 6 of the menstrual cycle, followed by 150 to 300 international units (IU) of human menopausal

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gonadotrophin (humagon (HMG) daily from 7 to 11 day of the cycle. The dose of the HMG was dependent on the response of the ovarian follicular development to the treatment protocol. The growth of the ovarian follicles was observed by ultrasonography and the determination of blood estradiole. When the follicular diameter was more than 18mm and the concentration of estradiole was 250pg/ml per follicle, ten thousand IU HCG were administered to induce ovulations. The intrauterine insemination (IUI) was performed 24 to 30 hours after HCG injection (1).

Sperm Activation: Blood was taken from the wife and the serum was separated and inactivated at 56C° in water bath for at least 30 minutes. First split ejaculated semen was allowed to liquefy at 37°C in an incubator. Modified Earl's culture medium supplemented with 285-290 mosml of 20% or 30% of inactive maternal serum, which was incubated at pH 7.4 under 5% of CO_2 air mixture, 98% humidity, 37°C temperature. This was used for sperm activation. Half to one ml of the first split ejaculate was mixed with 1.5ml of the culture medium supplemented with either 20% or 30% inactive serum. The mixture was centrifugated for 10 minutes at 400g (centrifugation force) and the supernatant layer was removed and the sperm pellet was layered with one ml of culture medium for 30 to 60 minutes. About 100 microliter of the top layer of sperms suspension in the activation tube was examined to record sperms analysis data. In case of simple layer technique for sperms activation, the same procedure was repeated apart from centrifugation - wash out step. The culture medium with 20% or 30% inactive serum was layered on the first split fraction of semen and incubated for 30 to 60 minute to allow sperm migration to top layer of the activation tube (12).

Intrauterine Insemination (IUI): A volume of 0.5 to 1.0 ml of the final sperms suspension was aspirated from the top layer of sperm activation tube by the transfer catheter under sterile conditions. The external cervical os was cleaned with fresh culture medium. The catheter was introduced into the uterine cavity via the cervical canal. Care was taken to avoid bleeding in the cervical canal wall during the passage of the catheter. The sperms were transferred to inside the uterus near the tubouterine junction. In cases of oligospermic patients, the volume of the culture medium was reduced to 0.3 ml. In cases of terato-spermic and asthenospermic males, only normal and active sperm cells in a volume of 0.20 ml containing at least 10 million sperm were used for IUI. Following sperm transfer, the patient remained in the lithotomy position for a period of 30 minutes. The presence of positive (3-HCG) test and demonstration of clinical symptoms of pregnancy were considered to establish pregnancy (13).

RESULTS:

It is obvious from the data of table (1, 2, 4, and 5) that the semen parameters were significantly improved ($P<0.001$) in their quality. At the same time, the activity of sperms (sperm motility and grade) was significantly improved ($P<0.05$), Table(3) following ***in vitro*** activation by using 30% serum and simple layer technique compared with 20% serum. However, all the sperm's parameters (concentration, percent of motility, grade activity and percent abnormality) had been significantly improved (P< 0.1, PO.05, PO.01, PO.05 respectively, Table 6) following *in vitro* activation by wash out technique using 30% serum in comparison with the same technique but with 20% inactivated serum.

Table 7 shows that the simple layer technique (using 20% inactive maternal serum) was found to be more efficient than centrifugation technique in improving significantly sperm concentration ($P<0.001$) and percentage of sperms' motility ($P<0.05$). The percentage of abnormal sperm morphology and grade motility was not significantly different between the simple layering and centrifugation techniques. The concentration of recovered sperms and sperms' motility were significantly higher in the simple layer technique compared to washout technique using 30% inactive maternal serum in the culture medium, ($P<0.005$,$P<0.05$ respectively Table (8).The grade activity of sperms and percentage of abnormal sperms' morphology in both techniques were not significantly different ($P>0.05$, Table 8).

The effect of the 20% and 30% inactive maternal serum on pregnancy rate per cycle following ***in vitro*** sperm activation by simple layering technique and intrauterine insemination is shown in table 9. Using 20% serum in the sperms activation medium and using it as a transfer medium resulted in 40.60% pregnancy rate per cycle versus 44.40% pregnancy rate per cycle using 30% inactive maternal serum. No significant differences were observed between the 20% and 30% in active maternal serum ($P>0.05$).

Discussion:

A significant abnormality ($P<0.05$) in the quality of semen in the present study was found in the age group of 39 to 49 years, and included semen volume, motility, morphology and sperm concentration. Other investigators reported that sperm production decreases as early as the third decade of life with reduction in ejaculate volume, percentage of viable and normal spermatoz,o, and the quality of seminal plasma (14).

Some of the factors which could be involved in the abnormality of sperm motility and morphology

are changes in seminal plasma osmolarity (15). In addition to this, the presence of human anti-sperm anti-bodies in the seminal plasma (autoimmunity) (16), or in the cervical or uterine fluids of the women (17), could play a significant role in male infertility.

In the present study, it was clear that using simple layering technique as compared to centrifugation technique for *in vitro* sperm activation with either 20% or 30% inactive maternal serum resulted in a significant improvements in sperm concentration and motility. Both sperm grade and abnormal sperm morphology were not significantly different in both techniques (P>0.05). The usage of centrifugation technique for *in vitro* sperm activation was found to have an adverse effect on sperm cells. The high centrifugation force (>400G) was found to reduce sperm viability and motility since non-viable sperms are unable to migrate to the top layer of the culture medium, which account for the reduction in sperm concentration in the centrifugation group (2,12).

Intra-uterine insemination (IUI) is used for the treatment of cervical factor (hostility), which is due to the presence of anti-sperm antibodies in the cervical mucous that inhibit sperm migration in the female reproductive system (2,18). In the present study, IUI was used to transfer the *in vitro* activated sperm near the site of oocyte fertilization in the fallopian tube by deposition of the sperm cells inside the cavity of the uterus and to avoid contact with cervical secretions. Using 30% inactive maternal serum for *in vitro* sperm activation and as a sperm transfer medium resulted in 44.4% pregnancy rate per cycle versus 40.6% pregnancy rate per cycle using 20% in active maternal serum. The difference between both groups was non-significant, which indicates that both concentrations have similar stimulatory effect on sperm activation. Al-Taee and Ridha-Barzanchi (12) reported 16% increase in sperm activity using 30% serum and 5% dextrose medium in 45 asthenospermic intefile men. In the present study, we observed 26% increase in sperm activity following *in vitro* sperm activation with 30% inactive maternal serum supplementing Earl's culture medium. The improvements observed in the present study may be due to the application of modified Earl's culture medium versus 5% dextrose solution. The modified Earl's culture medium contains calcium, amino acids, minerals and supplemented with nutritive substances and vitamins plus antibiotic, which have stimulatory effect on sperm activation *in vitro* and *in vivo* (2,19). Al-Anssari et al., (13) in 1993 reported 64% pregnancy rate per cycle following ovulation induction and IUI and luteal support therapy in infertile women with luteal phase defect versus 36% pregnancy rate in non-luteal support group. The higher pregnancy rate in that study versus 40.6% in the present study may be due to the fact that the infertility problem in Al-Anssari et al. work was mainly due to female infertility while in our study was due to male infertility. The 36% pregnancy rate per cycle in the non-luteal support group of women in Al-Anssari et al work is close to the reported 40.6% pregnancy rate in the present work. Ridha Albarzanchi et al. (20) reported 39.16% pregnancy rate per cycle using semen from 64 asthenospermic infertile men. They induced ovarian stimulation by clomiphene citrate and human menopausal gonadotrpin. In vitro sperm activation was performed followed by IUI. The reported 39.16% pregnancy rate per cycle agrees with data of the present work (40.6% pregnancy rate per cycle) and confirms our results.

It was concluded from the results of the present study that the use of either 20% or 30% inactive maternal serum in the culture medium, and the application of simple layering technique for human sperm activation in vitro followed by intrauterine insemination in stimulated cycle is an effective treatment in asthenospermic infertile patients.

Table (1):

Sperm parameters	Before activation*	After activation*
Concentration x10^6/ml	54.2 ± 4.64	21.46 ± 2.48**
Percent motility	38.5 ± 2.05	72.36 ± 1.99**
Grade motility	2.07 ± 0.07	314 ± 0.05**
Percent abnormal morphology	33.39 ± 1.57	17.29 ± 0.74**
Leucocytes and phagocytes concentration x10^6/ml	2.4 ± 0.28	None

The effect of sperm activation ***in vitro*** on sperm functions using 20% inactive maternal serum and simple layer technique following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
** P<0.001 significantly different from corresponding values. T-test for paired values

Table (2):

Sperm parameters	Before activation*	After activation*
Concentration x10^6/ml	54.85 ± 5.50	23.73 ± 3.20**
Percent motility	44.89 ± 3.86	78.50 ± 2.48**
Grade motility	2.29 ± 0.10	3.27 ± 0.05**
Percent abnormal morphology	34.14 ± 1.65	18.82 ± 1.10**
Leucocytes and phagocytes concentration x10^6/ml	2.67 ± 0.27	None

The effect of sperm activation ***in vitro*** on sperm functions using 30% inactive maternal serum and simple layer technique following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
** P<0.001 significantly different from corresponding values. T-test for paired values

Table (3):

Sperm parameters	Simple layer* 20%	Simple layer* 30%	Significance level
Concentration x10^6/ml	21.46 ± 2.48	23.73 ± 3.20	P<0.1
Percent motility	72.36 ± 1.99	78.5 ± 2.48	P<0.05
Grade motility	3.14 ± 0.05	3.27 ± 0.05	P<0.01
Percent abnormal morphology	17.29 ± 0.74	18.82 ± 1.10	P<0.05

The effect of simple layer technique on sperm activation ***in vitro*** using 20% versus 30% inactive maternal serum following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
Student T-test

Table (4):

Sperm parameters	Before activation*	After activation*
Concentration x10^6/ml	54.60 ± 5.28	10.70 ± 1.35**
Percent motility	41.25 ± 2.85	67.75 ± 2.11**
Grade motility	2.18 ± 0.08	3.15 ± 0.05**
Percent abnormal morphology	33.25 ± 1.82	15.75 ± 0.66**
Leucocytes and phagocytes concentration x10^6/ml	2.72 ± 0.34	None

The effect of sperm activation ***in vitro*** on sperm functions using 20% inactive maternal serum and wash-out technique following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
** P<0.001 significantly different from corresponding values. T-test for paired values

Table (5):

Sperm parameters	Before activation*	After activation*
Concentration x10^6/ml	61.88 ± 7.30	14.29 ± 1.72**
Percent motility	48.53 ± 4.64	72.94 ± 1.77**
Grade motility	2.26 ± 0.14	3.32 ± 0.05**
Percent abnormal morphology	33.82 ± 1.64	17.58 ± 0.87**
Leucocytes and phagocytes concentration x10^6/ml	2.67 ± 0.32	None

The effect of sperm activation in vitro on sperm functions using 30% inactive maternal serum and wash-out technique following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
** P<0.001 significantly different from corresponding values. T-test for paired values

Table (6):

Sperm parameters	Wash-out* 20%	Wash-out* 30%	Level of significance
Concentration x10^6/ml	10.7 ± 1.35	14.29 ± 1.72	P<0.1
Percent motility	67.75 ± 2.11	72.94 ± 1.77	P<0.05
Grade motility	3.15 ± 0.05	3.32 ± 0.05	P<0.01
Percent abnormal morphology	15.75 ± 0.66	17.58 ± 0.87	P<0.05

The effect of wash-out technique on sperm activation ***in vitro*** using 20% versus 30% inactive maternal serum following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
Student T-test

Table (7):

Sperm parameters	Simple layer* 20%	Wash-out* 20%	Significance level
Concentration x10^6/ml	21.46 ± 2.48	10.7 ± 1.35	P<0.001
Percent motility	72.36 ± 1.99	67.75 ± 2.11	P<0.05
Grade motility	3.14 ± 0.05	3.15 ± 0.05	P<0.05
Percent abnormal morphology	17.29 ± 0.74	15.75 ± 0.66	P<0.05

The effect of simple layer versus wash-out technique on sperm activation ***in vitro*** using (20% inactive maternal serum) following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
Student T-test

Table (8):

Sperm parameters	Simple layer* 30%	Wash-out* 30%	Significance level
Concentration x10^6/ml	23.73 ± 3.20	14.29 ± 1.72	P<0.005
Percent motility	78.5 ± 2.48	72.94 ± 1.77	P<0.05
Grade motility	3.27 ± 0.05	3.32 ± 0.05	P<0.05
Percent abnormal morphology	18.82 ± 1.10	17.58 ± 0.87	P<0.05

The effect of simple layer versus wash-out technique on sperm activation ***in vitro*** using (3% inactive maternal serum) following 30 minutes incubation period. Values are Mean ± SEM
* No. of patients per group = 28
Student T-test

Table (9):

Serum	No. of cycle pregnant (Percent*)	No. of cycle non-pregnant (Percent*)	Cycle number
20%	13 (40.6)	19 (59.4)	32
30%	16 (44.4)	20 (55.6)	36
Total	29 (42.6)	39 (57.4)	68

The effect of 20% and 30% inactive maternal serum on Pregnancy rate following intrauterine insemination with hyperactive serum prepared by simple layer technique.
P>0.05 (Chi-square test)

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