ORIGINAL RESEARCH ARTICLE

Low Cost, Simple, Intrauterine Insemination Procedure with Unwashed Centrifuged Husband's Sperm for Developing Countries

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Abstract

There is an increased need for low cost procedures in treating infertility particularly in developing countries. Intrauterine insemination was used long before the advent of in vitro fertilization. During the last 30 years however, intrauterine insemination has evolved with the introduction of ovulation stimulating protocols and sperm preparation methods taken from assisted reproduction techniques. Costs have risen, but the success rate has not risen to the same extent. We have therefore developed a quite simple intrauterine insemination technique which may be performed in developing countries, without the need of sophisticated equipment, costly materials, media, or disposable insemination catheters; it is quite inexpensive and may be performed by trained staff, such as nurses or midwives. 20 to 27 % (depending on the aetiology of their reproduction problem) of the couples remained clinically pregnant after an average of 3.5 to 3.8 intrauterine inseminations procedures. (*Afr J Reprod Health 2012; 16[4]: 175-179).*

Résumé

Il y a un besoin croissant de procédures à bon marché dans le traitement de la stérilité surtout dans les pays en développement. L'insémination intra-utérine a été utilisée bien avant l'avènement de la fécondation in vitro. Au cours des trente dernières années, toutefois, l'insémination intra-utérine a évolué avec l'introduction des protocoles qui stimulent l'ovulation et les méthodes de la préparation des spermatozoïdes empruntés par les techniques de procréation assistée. Les coûts ont augmenté, mais le taux de réussite n'a pas augmenté de la même manière. Nous avons donc mis au point une technique d'insémination intra-utérine assez simple qui peut être effectuée dans les pays en développement, sans avoir recours aux équipements sophistiqués, aux matériaux coûteux, aux médias, ou aux cathéters d'insémination jetables. Il est assez bon marché et peut être réalisé par le personnel qualifié, comme les infirmières ou des sages-femmes. 20% à 27% (selon l'étiologie de leur problème de reproduction) des couples sont restés cliniquement enceintes après une moyenne de 3,5 à 3,8 procédures d'insémination intra-utérine (*Afr J Reprod Health 2012; 16[4]: 175-179).*

Keywords: Intrauterine insemination, simple, IUI, low cost, sperm, unwashed

Introduction

Assisted reproduction techniques, particularly In Vitro Fertilization (IVF) and even more so Intra Cytoplasmic Sperm Injection (ICSI), are quite expensive and require sophisticated procedures. In fact they need sophisticated equipment, specialized laboratory staff, disposable material, culture media, specific drugs for stimulating ovulation.

Intrauterine insemination (IUI) with husband's sperm as it is usually performed nowadays is less expensive than IVF, but still requires disposable materials, media with short expiry dates and it is frequently performed in association with the daily administration of human menopausal gonadotropins (HMG) for approx. 10 days to induce the growth of several ovarian follicles.

In many developing countries there are no or very few centers capable of offering sophisticated technologies such as IVF/ICSI. Also, it is often difficult to find locally HMG and specific media for sperm preparation. In addition, contrarily to many developed countries, assisted reproduction is not normally offered by social or government organizations, since there are other more stringent priorities and costs of such techniques are so high that very few people can take advantage of these procedures. On the other hand, to be infertile in developing countries is a social and psychological

nightmare, more serious than in developed countries. Therefore we have developed a low cost procedure: the Simplified Intra Uterine Insemination (SIUI), which may be applied to many infertility cases and may be feasible quite easily in developing countries. Of course, tubal blockage, very poor sperm quality, reduced or absent ovarian reserve cannot take advantage from SIUI.

We have tried our best to reduce costs to a minimum, trying to obtain an acceptable success rate and keeping the complication rate as low as possible.

SIUI is a technique with a yield of success lower than IVF/ICSI, but may be repeated many more times, especially if it is simple and the cost is kept very low. Thus, at least part of the infertile population may find a solution to their problem with such a simple and affordable procedure.

Methods

Patients selection

Infertility of at least 2 years duration. The higher age limit for females was 43 and 60 for males.
Both males and females had to be free of HIV, B and C Hepatitis, Syphilis. In the male, the sperm had to meet the following minimal criteria: two million motile spermatozoa in the whole ejaculate, even if the motility was sluggish.
In the female, a previous hysterosalpingography showing patent tubes, and regular cycles or in case of irregularities in FSH value not higher than 15 I.U. /l .

Ovulation stimulation and monitoring

Ovulation was stimulated by administering to the female patients Clomiphene citrate (CC) 100 mg daily orally for 5 consecutive days, starting on cycle day 4. Follicular growth was monitored by ultrasound with a vaginal probe. Monitoring started on day 9 and thereafter every other day. Human Chorionic Gonadotropin (HCG) 5,000 IU were administered only once by intramuscular injection when the leading follicle had reached a mean diameter size of 18/19 mm. SIUI was performed 36 hours after the HCG injection. No supplementation was given during the luteal phase.

Sperm preparation

Husband's raw sperm was left 15 minutes undisturbed after ejaculation in a 50 ml glass jar, at room temperature, for liquefaction. It was then poured off directly into a glass, 10 ml, centrifuge test tube. It was then centrifuged at 2,500 r.p.m. for 3 or 4 minutes, with a small bench centrifuge. The supernatant was completely discarded by simply turning the tube upside down (care was taken not to leave any fluid part at all in the test-tube). The pellet remained stuck, almost dry, at the bottom of the test-tube. Using the tip of a metallic intrauterine catheter the pellet was quickly stirred and aspirated into the catheter, by an insulin syringe connected to the catheter. The volume of the pellet normally did not exceed 0.2 ml.

Intrauterine insemination

The insemination was performed immediately, using a fine metallic catheter having a small bend, 3 cm from its tip and a small olive shaped tip to facilitate passage into the uterus. The catheter was gently introduced, in difficult cases with the help of a tenaculum, into the uterine cavity and the pellet was injected. Patients were advised to step down of the gynaecological bed right away and to resume their normal activities. They were also asked to report in case of any complication and to come back to the clinic 3 to 4 weeks after the insemination.

Results

This study lasted 12 months, from the 1st of July 2009 to the 30th of June 2010. Fifty five couples met the selection criteria and were enrolled in the study. In 4 cases no follicle developed as seen by ultrasound, and these cases were discarded. The remaining 51 couples were divided into 2 groups according to the type of sperm motility: Group A having a rapid, progressive motility of 10% or higher (22 couples); Group B having a progressive motility lower than 10% or a sluggish motility (29 couples). Group A performed a total of 79 SIUI

(an average of 3.6 procedures per couple) and obtained 6 clinical pregnancies: all were singleton pregnancies except one which was a twin pregnancy. The clinical pregnancy rate was 7.6% per SIUI and 27% per couple. Group B performed a total of 110 SIUI (an average of 3.8 procedures per couple) and obtained 6 clinical pregnancies: all were singleton pregnancies. The pregnancy rate was 5.5% per SIUI and 20.6% per couple. No signs or symptoms of infection were detected, no hyperstimulation occurred and no abdominal contractions were reported. In a few cases some patients reported abdominal discomfort, bloating or mild spotting, but this was not dissimilar to what happens even with standard IUI, when HMG is used or when the sperm is prepared with conventional swim-up or other more sophisticated sperm preparation procedures.

The overall cost of the SIUI procedure, excluding the doctor fees, was equivalent to approx. 16 US dollars (7 US dollars for the CC tablets and 9 US dollars for the HCG 5,000 IU injection), whereas in the IUI conventional procedure, using HMG, sperm preparation media and disposable materials, the costs may rise to not less than approx. 399 US dollars (350 US dollars for HMG, if 150 IU are used daily for 10 days, which is usually the minimum requirement, 9 US dollars for HCG 5000 IU, 20 US dollars for the media and 20 US dollars for the disposable catheter and other plastic disposable materials).
If on the other hand one performs conventional IUI in association with CC/HCG stimulation, but uses media and disposable material, the cost will be approx. 56 US dollars (16 dollars for the drugs and 40 dollars for media, disposable catheter and other plastic materials).

Discussion

With the method depicted here, a high number of spermatozoa is placed into the uterine cavity, as compared to common techniques of sperm preparation such as swim up or gradient centrifugation[1], since we did not do any effort to separate and use the best spermatozoa, the most motile or those with the best morphology. Using a rather high speed centrifugation, only a small amount of spermatozoa remained in the seminal plasma and was discarded while most of the spermatozoa were concentrated into the pellet. The fact that motile, immotile spermatozoa and cellular debris are inseminated together does not seem to prevent pregnancies since the motile spermatozoa accomplish their duty whether there are immotile spermatozoa and debris around or not; however the production of reactive oxygen species was of concern to us[2]; this is why we limited the centrifugation time to 3 or 4 minutes and performed the insemination as soon as the centrifuge stopped. The pellet would thus immediately spread and dilute into the uterine cavity, reducing the possible harmful effects of these compounds. Moreover, the uterus is characterized by a continuous flow of fluid which helps sweep away these compounds[3]. This is quite different from what happens in vitro where the environment is still and is renewed only by changing the medium.

However the question concerning reactive oxygen species is not at all clear, since these compounds may even be useful in triggering or facilitating capacitation in human sperm[4].

No signs or symptoms of infection were detected; we did not search for the presence of bacteria in the whole ejaculate or in the centrifuged pellet, but one may speculate that the short centrifugation time may not allow for a massive migration of potential bacteria in the ejaculate to the bottom of the centrifuge tube. In fact bacteria commonly have a small mass and volume as compared to spermatozoa and therefore their sinking speed during centrifugation would be slower; therefore, their presence in the pellet may not reach the load threshold needed to give rise to infections. In addition, the lack of uterine contractions indicates that prostaglandins were almost completely discarded with the seminal plasma and those confined within the pellet were not enough to give rise to contractions. Before the assisted reproduction era, IUI were usually performed using whole semen and uterine contractions were one of the major complications[5]. In the present study, we cared that all the seminal plasma was discarded in order to eliminate possible complications.

The results obtained with SIUI are quite satisfactory, since our unsuccessful patients may

undergo a few more SIUI attempts and some other pregnancies may still be obtained. Pregnancies reach a plateau after 6 IUI attempts in male infertility cases and pregnancies may still be achieved after 6 attempts in idiopathic infertility[6] even though the greatest numbers of pregnancies are usually obtained during the first attempts.

It is interesting to observe that 6 pregnancies developed in Group B, characterized by poor semen samples. Many authors observed that patients with poor semen are not candidate for IUI: this may be in part due to the fact that semen preparation techniques cause a reduction of the spermatozoa concentration since a selection is made and only the most motile spermatozoa are inseminated; however the selection process per se may involuntarily discard many potentially fertile spermatozoa. With the described technique no selection is performed and almost all the ejaculated spermatozoa are inseminated. In fact, even though motility and morphology rather than concentration are by far the most important factors for obtaining a pregnancy, there still is the need for a minimal concentration which may not be achieved in borderline cases when a method of selection of spermatozoa is applied. In addition, recently some authors observed that preparation of spermatozoa on discontinuous density gradients may even be harmful since it may aggravate oxidative stress by stimulating DNA-base adduct formation[7].

Differently from in vitro fertilization techniques, where the spermatozoa need to be completely freed of the seminal plasma, since this fraction of the ejaculate contains factors inhibiting capacitation[8], with the described technique capacitation does not need to occur outside the body, but takes place in the female genital tract. In fact, since the advent of IVF, thirty years ago, IUI has undergone a change in the way sperm is being prepared. The sperm undergoes now in vitro capacitation procedures similar to those applied to IVF. However, since situations differ very much whether they occur in vitro or in vivo, it is tempting to speculate that the process of capacitation of the sperm in vitro may differ from capacitation in vivo. It would be interesting to compare IUI results in a group having the sperm capacitated in vitro and a group with capacitation in vivo as in the present cases.

The cost of the described, CC/HCG stimulated, low cost procedure (doctor fees excluded) is 3.5 times less than conventional, CC/HCG stimulated IUI using media and disposable materials and 25 times less than HMG stimulated IUI associated with the use of media and disposable materials. In addition, since HMG as well as sperm media are used quite sporadically in developing countries, they are often difficult to find and have to be ordered directly from abroad. During transportation, which may take a few days, they must be kept at low temperature and this condition is not always complied with. Moreover, in certain countries, importation laws make it difficult to obtain these items, particularly HMG. And in any case a quite large survey on IUI[9] has shown that IUI yields higher results if the cycle is stimulated with CC rather than with gonadotropins.

Of course, the indications for IUI are limited as compared to the indications for IVF and ICSI, but the simplicity, the repeatability and the very limited cost makes SIUI a useful tool for the treatment of at least part of the infertility cases, especially in developing countries for patients with quite small revenues.

The technique may be learnt and performed not only by gynecologists (these specialists are quite few in developing countries), but also, where the law permits, by general practitioners, mid-wives, specialized nurses. The materials used may be washed, sterilized and reused indefinitely. The need for equipment is very limited: an optic microscope for the sperm, a small bench centrifuge and a dry heat sterilizer or an autoclave, all items normally present even in small clinics and dispensaries.

Ultrasound machines have not yet spread everywhere in developing countries; therefore we have recently started a case control study where a group of patients will be submitted to SIUI without ultrasound monitoring as opposed to a group whose ovulation will be monitored by ultrasound. We have observed in fact that using CC, ovulation may be triggered by HCG injection in the same day of the cycle for most of the patients. Moreover, IUI may be performed on the day of HCG administration, seemingly with better

results than with the conventional 36 hours delay after HCG[10]. All this probably allows for quite a flexibility in the timing of insemination. The need for lowering costs of assisted reproduction techniques is stringent particularly in developing countries where revenues are very low, governments cannot provide for these treatments and, for cultural and social reasons, infertility is felt as a blame, females are often unfairly accused of being responsible and marriages are being disrupted.

Contribution of Authors

The study was conceived and designed by Leonardo Formigli. The manuscript was also prepared by him. The clinical work as well as the collection and analysis of data were performed by Rose Nsa Ada Mve. All authors mentioned in the article approved the manuscript.

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