Journal of Assisted Reproduction and Genetics, Vol. 21, No. 4, April 2004 (© 2004)

Male Infertility

Parameters Affecting the Results in a Program of Artificial Insemination With Donor Sperm. A 12-Year Retrospective Review of More Than 1800 Cycles

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Submitted April 21, 2003; accepted February 27, 2004

***Purpose*:** We aimed to establish the influence of the parameters affecting artificial insemination (AI) results in order to describe the ideal situations to achieve the best results as well as to adequately counsel the patients undergoing these treatments about their pregnancy chances.
***Methods*:** We performed a controlled retrospective clinical study over more than one decade in a total of 1858 cycles in 710 patients. Clinical histories and computer registers were systematically reviewed between January 1990 and June 2002. We analyzed the influence of diverse factors affecting AI results such as patient's age, ovarian stimulation, and seminal characteristics to offer a detailed description of the technique.
***Results*:** Less than 35-years-old, smooth ovarian stimulation and 5 million of progressive motile sperm inseminated two consecutive days are the optimum conditions for achieving good results. Also, period of time that sperm remained frozen do not affect the result. Furthermore, we present the likely or expected outcomes of these treatments depending on the male and female etiologies.
***Conclusions*:** We discourage AI in aged patients, and strongly recommend undergoing ovarian stimulation. Nonetheless, we must reach an adequate amount of sperm with good motility in order to inseminate with maximum guaranties of success.

KEY WORDS: Age; artificial donor insemination; motile sperm; ovarian stimulation; retrospective.

INTRODUCTION

Artificial insemination (AI) with the use of semen from anonymous donors has been a low-complexity assisted reproduction technique widely used for many years in the treatment of women wishing to conceive in specific situations.

The indicators for the employment of donor sperm are severe male factors (SMFs) both in patients with very low or absence of spermatogenesis and patients with sexually transmitted infectious diseases (where the male must have sexual intercourse with protection, thus avoiding the infection to the partner, as in human immunodeficiency virus) (1), and also in males with genetic disorders that might be transmissible to the progeny, as well as homosexual women and women without a male partner.

The introduction of intracytoplasmic sperm injection (ICSI) to the assisted reproduction laboratory has notably decreased the number of AI over recent years, mainly in patients showing severe alterations of the spermatogenesis (author's own data).

In other cases, we expect a reduction in the use of donor sperm as new techniques become developed or when other techniques become firmly established,

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1058-0468/04/0400-0109/0 © 2004 Plenum Publishing Corporation

for instance, preimplantation diagnosis of genetic diseases as well as sperm washing for HIV serodiscordant couples (1).

Hence, it is essential to determine the relevant factors affecting the results of AID programs, and in this way we can establish the adequate criteria to counsel, estimate success prognosis, and improve success rates.

Different studies have described the influence of different parameters such as maternal age, male etiology, female etiology, ovarian stimulation protocol, and many others with controversial or rather diverging results.

A number of studies with adequate sample sizes were conducted from different clinics together in multicenter studies, where probably the selection criteria, control, and management of patients are slightly different, thus adding heterogeneity to the sample.

By means of this study, our aim was to review our experience spanning more than 12 years with almost 2000 AI cycles done in about 500 women in a unique setting to determine the factors that influence the success of this technique.

MATERIALS AND METHODS

Clinical histories were systematically reviewed, together with all the computer registers of each AI performed in the period between January 1990 and June 2002 at the Instituto Valenciano de Infertilidad in Valencia.

The factors analyzed were as follow: woman's age, cycle number, diagnosed male infertility, ovarian stimulation protocol, presence/absence of pregnancy, multiple pregnancies, multifetal pregnancies, ectopic pregnancies, abortions, time that sperm remained frozen until used and the total number of inseminated sperm with progressive motility.

A total of 1858 cycles in 710 patients were included in our work. It is important to remark that every patient was not included in every parameter studied because of data loss or incomplete registers.

Regarding age, each patient was included into a category depending on the moment of the insemination. We performed a number of cycles which varied in each woman from 1 to 6. After the analysis of the raw semen sample and a subsequent centrifugation, sampling of the pellet, and extensive search, azoospermic patients were considered to be those males with no sperm in the ejaculate in at least three consecutive spermiograms.

Males with infectious diseases and those considered anejaculatory were not considered azoospermic although probably their partners never had any sperm exposition to spermatozoa.

The different ovarian stimulations historically carried out in these cycles, in order to increase the number of mature oocytes available, can be categorized into four groups: natural (NC), clomiphene citrate (CC), human menopausal gonadotrophin (hMG), and follicle-stimulating hormone (FSH) cycles.

For insemination frozen sperm was always employed after the corresponding quarantine that allowed the confirmation of the absence of infectious diseases in the donor (confirming this fact with a blood analysis at least 6 months after the sample donation and freezing). Samples were subsequently thawed when needed after their selection to match the couple's phenotypical characteristics as well as blood type, as stipulated in the Spanish Assisted Reproduction Law.

Semen characteristics were analyzed in the samples after thawing as stated in the WHO manual for motility and concentration. All the donor samples were adequately studied during their donation period by means of periodical analysis in our own center to eliminate the presence of sexually transmitted diseases and genetic disorders.

The complete list of serological determinations, as well as the frequency of finding positive results on the serologies has been analyzed in detail by our group, demonstrating that this frequency in our sperm donor population is not greater than that present in infertile couples or in the general population (2).

Over the last few years different freezing protocols have been used mainly based on glycerol and egg-yolk; nonetheless, we have previously demonstrated that no differences between these two methods affect either postthaw semen survival and motility or pregnancy rates achieved (3).

In regard to semen capacitation, density gradients have been purchased from different commercial sources, including classical Percoll® and PureSperm®, although a few swim-up procedures were performed. Our own unpublished data also confirm that no differences among capacitation methods exist.

Depending on stage of technical development, two consecutive intrauterine inseminations were done with different catheters (mainly Vygon® and Gynetics®), approximately 12 and 36 h after the 5000 IU human chorionic gonadotropin (hCG) administration. Again, the use of different catheters does not affect the results (author's own data).

Table I. Overall Results on Both Natural and Stimulated Cycles

Cycle number	Number of cycles	Pregnancy rate (%)	Cumulative pregnancy rate (%)
1	654	20.1	20.1
2	480	20.7	36.6
3	295	20.4	49.6
4	216	16.2	57.8
5	128	16.5	64.8
6	85	21.1	72.3
Total	1858	19.1	

Note. $p > 0.05$.

Pregnancy tests were done at Day 14 after the first insemination by the quantification of hCG, and 7 days later confirmed by vaginal ultrasonography. Abortions were considered as those pregnancies spontaneously interrupted before the 20th week.

The data were grouped into life tables to visually represent and compare groups.

The statistical tests applied were Mantel–Cox chi-square or *t* test with ANOVA and post hoc Tests (Bonferroni, Scheffé, and DMS), where appropriate.

RESULTS

A total of 1858 cycles in 710 patients were performed over a period of 12 years, yielding an approximate rate of 154 AI/year. A total of 364 pregnancies were achieved giving a global pregnancy of 19.7% per cycle and 51.3% per patient. The accumulative global pregnancy rate at the 6th cycle was 72.3%. The patients' age ranged between 22 and 46.

In addition, there were 168 abortions, giving an abortion rate of 9.1% per cycle. Also, there were, within the stimulated patients, 66 multiple pregnancies (two sacs, 3.6% per cycle) and 35 multifetal (three or more, 1.9% per cycle).

Table II. Pregnancy Rate Per Patient in a Program of AI With Ovarian Stimulation

Cycle number	Cycles (pregnancies)	Pregnancy rate (%)	Cumulative pregnancy rate (%)
Natural cycles			
1	153 (21)	13.7	13.7
2	119 (12)	10.1	22.4
3	84 (11)	13.1	32.6
4	52 (6)	11.5	40.4
5	29 (2)	6.9	44.5
6	21 (3)	11.1	52.5
Total	458 (55)	12.0	
Stimulated cycles (CC, hMG, FSH)			
1	489 (105)	30.1	30.1
2	349 (85)	24.4	47.1
3	242 (49)	20.3	57.8
4	163 (29)	17.8	65.3
5	94 (18)	29.8	75.7
6	63 (15)	23.8	81.5
Total	1400 (301)	21.5*	

Note. $p > 0.05$ among cycle number; $^{*}p < 0.001$.

Only 11 ectopic pregnancies were found, resulting in a rate of 0.6% per cycle which resulted in pregnancy, and 0.03 per cycle attempted.

Pregnancy rates per cycle and cumulative pregnancies are detailed in Table I. There are no statistical differences between the number of cycle and the results.

Ovarian Stimulation

In relation to ovarian stimulation, only patients with a unique stimulation protocol were included, thus excluding mixed protocols with combinations of gonadotrophins within the same cycle. Results are expressed in Tables II and III.

Table III. Pregnancy Rates Per Patient in a Program of AI With Ovarian Stimulation

	Ovarian stimulation with clomiphene citrate			Ovarian stimulation with hMG			Ovarian stimulation with FSH		
Cycle number	Number of cycles	Pregnancy rate	Cumulative pregnancy rate	Number of cycles	Pregnancy rate	Cumulative pregnancy rate	Number of cycles	Pregnancy rate	Cumulative pregnancy rate
1	32	15.6	15.6	179	20.1	20.1	270	24.1	24.1
2	26	11.5	25.4	131	25.2	40.2	195	25.6	43.5
3	21	9.5	32.5	89	16.9	50.3	130	24.6	57.4
4	16	12.5	40.9	50	12.0	56.2	94	21.3	66.5
5	13	15.4	50.0	38	13.2	62.0	47	25.5	75.1
6	11	18.2	59.1	18	16.7^{+}	68.3	30	30.0	82.5
	119	13.4		495	19.7		746	25.2†	

Note. $p > 0.05$ among the number of cycle; $^{\dagger}p < 0.01$; $^{+}p < 0.001$.

In the first, we can observe that AI reaches better results, almost doubling pregnancy rates when the ovaries are stimulated in comparison with NC (21.5% vs. 12.0%), being those differences statistically confirmed ($p < 0.001$). Then, the cumulative pregnancy rates in the first is higher than 80% in 6 cycles, while only 52.5% is reached in the last, without a moderate stimulation of the ovaries. Furthermore, we can see important differences depending on the substance employed during the stimulation.

When they were compared, pregnancy rates obtained by using FSH in the ovarian stimulation were higher than those obtained by the use of hMG (25.2% vs. 19.7%; $p < 0.01$), and the latter, higher than CC (13.4%; $p < 0.01$). Cumulative rates at the 6th cycle are notably higher in the group of FSH reaching 82.5%. On the other hand, no differences were obtained when the results of hMG and CC stimulations were compared.

Also another interpretation of the results can be made by comparing the results of stimulated versus nonstimulated cycles. This comparison can be found in Fig. 1, where it is shown that pregnancy rates are 1.7 times higher in stimulated cycles than in nonstimulated ones.

Multiple pregnancies are an important aspect of ovarian stimulation that needs attention. In this study, the multiple pregnancy rate was only 1.4% in spontaneous ovulatory cycles (NC), 1.6% in CC cycles, 3.5% in hMG cycles, and 5.5% in FSH cycles; statistical difference not being significant between them.

From all this information, we can conclude that ovarian stimulation with FSH is by far the recommended therapeutic option in the AI treatments.

Male Pathology

Another unknown feature to be revealed by this work was the influence of the male pathology on the AI results, which are obtained depending on the AI indication.

In Fig. 2, we can see the differences regarding male factors. Prognoses were best for the ones with the presence of genetic alterations potentially transmissible to the progeny and the couples undergoing AI after repeated ICSI failure.

With pregnancy rates per cycle approaching 75 and 80% (both statistically different from the remaining groups; $p < 0.01$ taken as between ICSI failure and serodiscordant couples, and $p < 0.001$ between the two cases with the best results in relation to the remaining groups). In the other cases, results were quite similar to the general mean.

We further analyzed the differences between couples whose male partner presented some degrees of spermatogenic function, i.e. where spermatogenesis was severely impaired, but where sperm can be retrieved with the following characteristics: less than 5 mil/ml, 5% normal forms, or 20% with progressive motility. The results can be observed in Tables IV and V, also as a function of the hormonal treatment received.

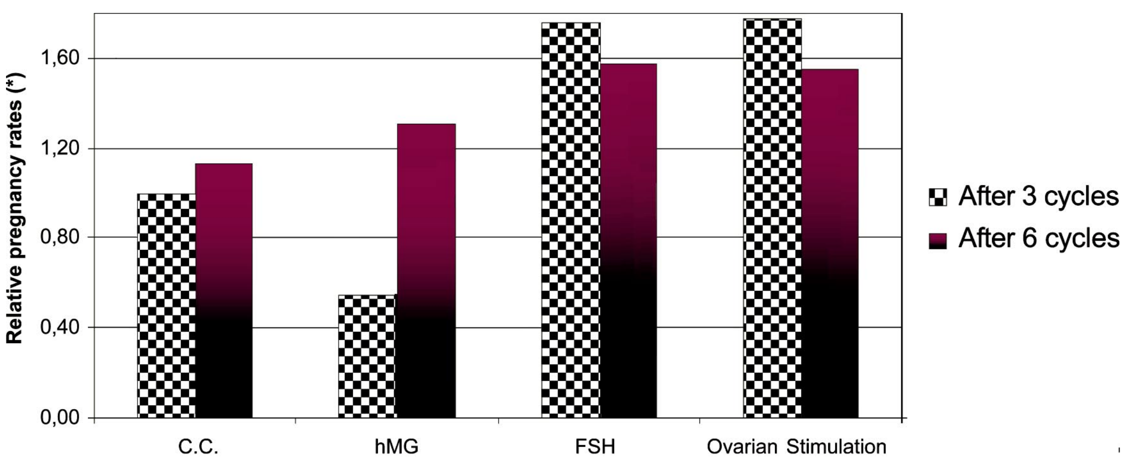

Fig. 1. Relative pregnancy rates in an AI program. Results obtained with ovarian stimulation with CC, hMG, and FSHhp including only natural cycles (NC). The symbol "*" indicates results of dividing cumulative pregnancy rates after 3 or 6 cycles of the group with stimulation or NC.

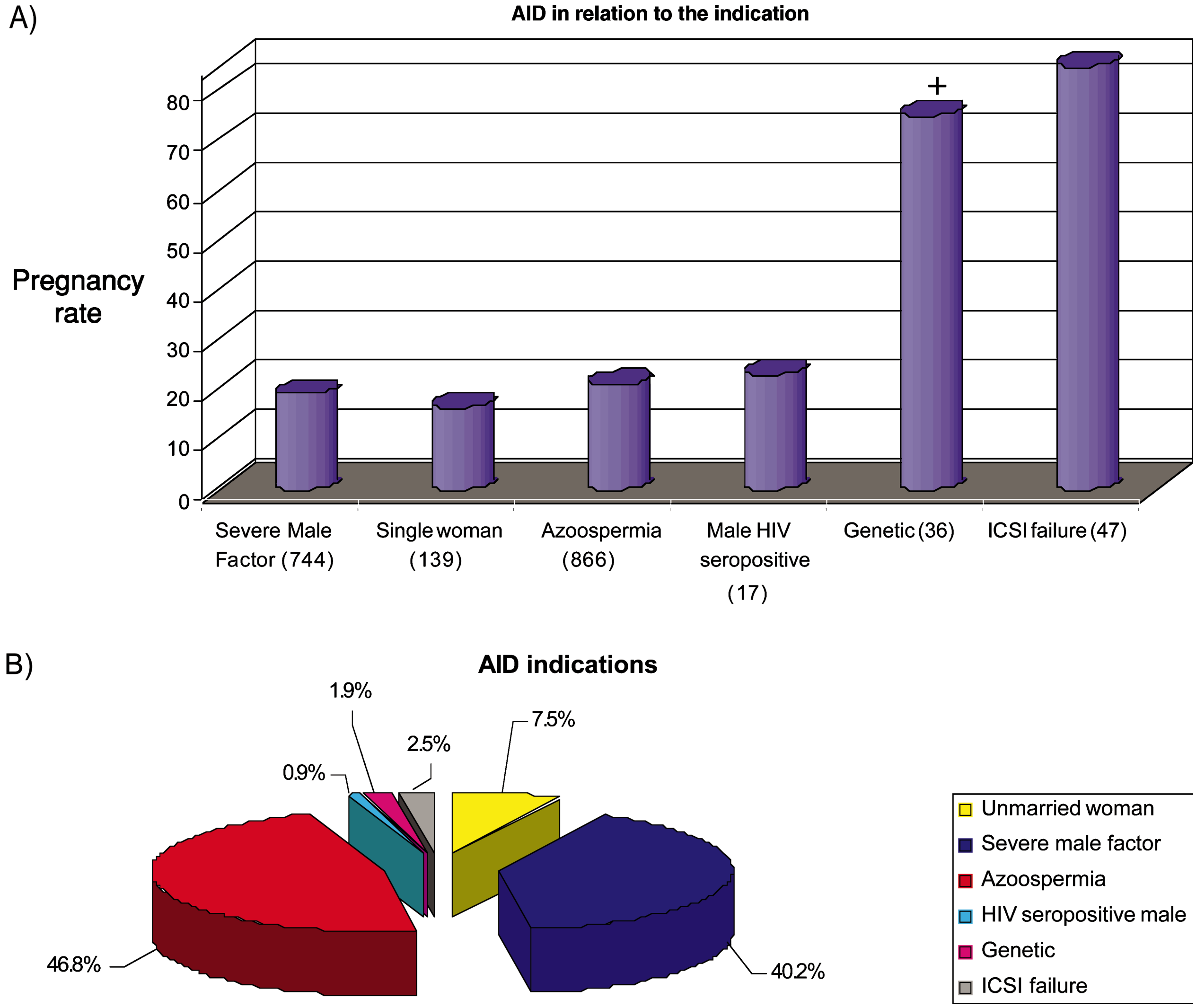

Fig. 2. AI results in relation to the indication. (A) $^{+}p < 0.001$ between Genetic or ICSI failure and the remaining. (B) $^{\dagger}p < 0.01$ between HIV seropositive male and ICSI failure.

Table IV. Pregnancy Rates Per Cycle and Cumulative Situation in Azoospermic Patients or With Severe Male Factor and Natural Cycles

	Azoospermia and natural cycles			Severe male factor and natural cycles		
Cycle number	Number of cycles	Pregnancy rates	Cumulative pregnancy rates (%)	Number of cycles	Pregnancy rates	Cumulative pregnancy rates (%)
1	83	18.7	18.7	61	6.6	6.6
2	64	14.1	29.6	51	5.9	12.2
3	44	18.2	42.4	36	8.3	19.4
4	21	14.3	50.6	29	6.9	25.0
5	16	12.5	56.8	12	0.0	25.0
6	12	16.7	64.0	10	10.0	32.5
Total	240	16.5*		199	6.5*	

Note. $p > 0.05$ among the cycle number; $^{*}p < 0.01$.

Table V. Pregnancy Rates Per Cycle and Cumulative Situation in Patients With Severe Male Factor or Azoospermia in a AI Program With Ovarian Stimulation

	Azoospermia and stimulated cycles			Severe male factor and stimulated cycles		
Cycle number	Number of cycles	Pregnancy rates	Cumulative	Number of cycles	Pregnancy rates	Cumulative
1	213	22.4	22.4	196	20.6	20.6
2	157	24.7	41.6	136	29.7	44.1
3	120	26.4	57.0	90	20.8	55.7
4	75	22.2	66.6	63	21.6	65.3
5	46	26.7	75.5	38	15.4	70.6
6	35	20.0	80.4	22	22.7	77.3
Total	646	22.3		545	20.1	

Note. $p > 0.05$ among cycle number.

When results were compared between couples with azoospermic males and couples with SMF in relation to pregnancy rates per cycle and only considering nonstimulated cycles, we find significantly higher rates in the cases of azoospermia, $p < 0.01$. Obviously, both groups displayed better results in the stimulated cycles when they were compared with the NC ($p < 0.01$).

Surprisingly, it seems that ovarian stimulation tends to equal the results between groups, being statistically comparable ($p < 0.05$). The differences are consequently much more pronounced in the NC than in the stimulated cycles (16.5% against 6.5% in the former and 22.0% against 20.1% in the latter, respectively).

Age

Another important factor that we analyzed, age, has also been demonstrated to be relevant for AI results. This can be concluded from the analysis of the results as a function of the age group where the women were included. Again, we also considered the kind of stimulation they received. Results are detailed in Fig. 3 and Table VI.

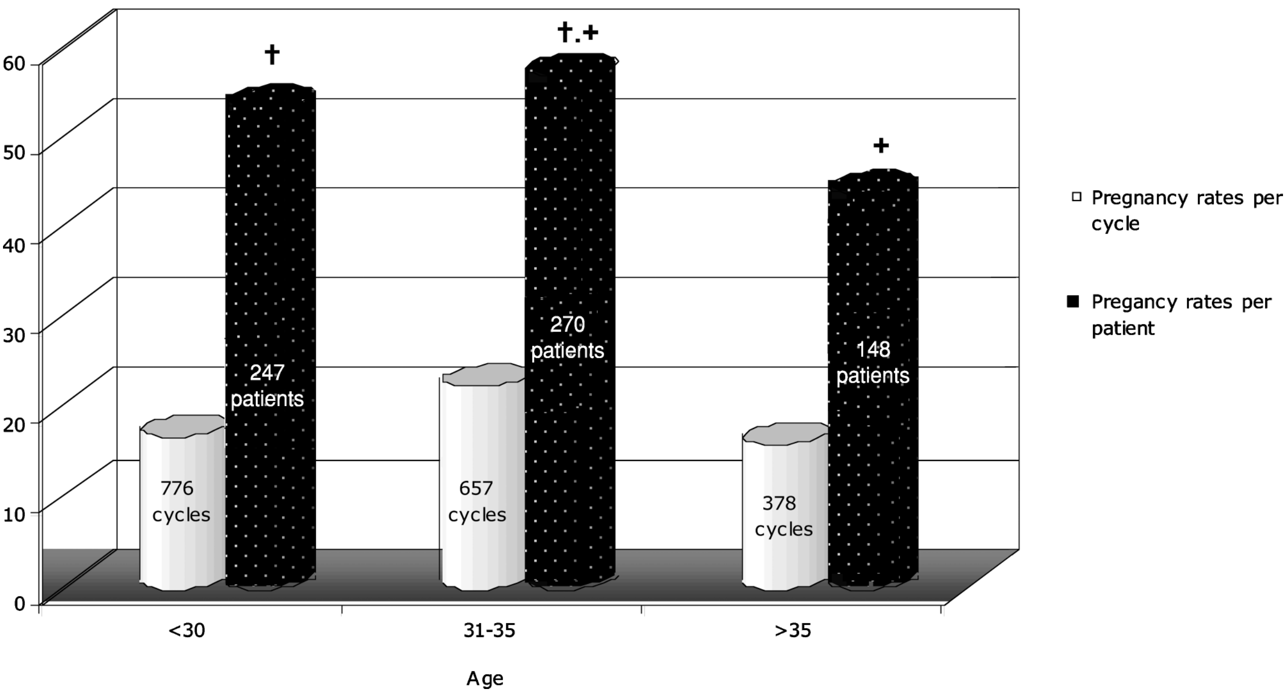

Fig. 3. Influence of age on pregnancy rates per patient in AI between natural and stimulated cycles. $^{\dagger}p < 0.01$, $^{+}p > 0.05$.

Table VI. Age Influence Over Pregnancy Rates Per Cycle and Patient in AI on Stimulated and Natural Cycles

	Natural cycles			Stimulated cycles		
Age	Cycles (patients)	Pregnancy rates/cycle	Pregnancy rates/patient	Cycles (patients)	Pregnancy rates/cycle	Pregnancy rates/patient
<30 years	630 (197)	17.9	57.0	146 (50)	13.0	38.0
31–35 years	452 (158)	27.6*	79.1	205 (69)	11.7	34.7
>35 years	274 (58)	18.2	86.2	104 (35)	9.5	31.4
Total	1356 (413)	21.2	69.7	455 (154)	11.8	35.0

*$p < 0.001$.

Three different groups were preestablished: up to the age of 30, from 31 to 35 years, and from 36 years onwards, which has previously been set as the age limit where the female reproductive function remains adequate.

In the Fig. 3, we can appreciate a clear drop in the global results in women aged >35, although, surprisingly, these results do not differ significantly from those obtained in women aged <30. The best results are obtained in women whose age ranged between 31 and 35 years, and this segment was statistically significant ($p < 0.001$) when compared with any other group.

When we consider the stimulation protocol or NC, pregnancy rates per cycle in any age group, the results give appreciably higher values in gonadotrophin-stimulated women, and is more evident as age increases (Table VI). Again, it seems that ovarian stimulation is able to improve the results of the worse group until the results begin to be comparable.

Donor Sperm Cryopreservation Time

Another retrospective analysis of our results was carried out with the aim of determining the influence of the time that the sperm samples have been maintained frozen over the sperm fertilizing potential (Table VII). To this end, we have solely taken into account those data coming from the use of one unique sample (obviously from the same donor) that allowed a sample size of 416 AI. We discarded for this study those AI performed with two different semen samples from the same donors that were frozen on different days.

The results clearly show that sperm properties are maintained throughout the whole period of time. It was statistically demonstrated that the time that sperm has been frozen has no negative influence on either the pregnancy rates or in the abortion rates. Other factors influencing this study were comparable, such as total motile sperm inseminated, women's mean age. No differences between groups were found on them.

Number of Progressive Motile Sperm Inseminated

The final analysis of our results was performed to compare the influence of total motile progressive sperm inseminated either in the first, second, or both days of insemination, separately or together. We undoubtedly demonstrated that more than 5 million (mil) total motile sperm inseminated in as many days as possible are needed to reach the best pregnancy rates (Table VIII).

In any case, this data requires further corroboration, since the low number of AI included up till now (particularly when a pattern of both days of insemination is studied) means that the results can only be statistically powerful when isolated days of insemination (either the first or the second) are considered.

Table VII. Influence of the Time That Sperm Was Kept Frozen in AI Results

Time	Between 6 months and 1 year ($n = 118$)	1–1.5 years ($n = 109$)	1.5–2 years ($n = 96$)	2–2.5 years ($n = 45$)	More than 2.5 years ($n = 48$)	Total ($n = 416$)
Women age (years)	33.3 ± 0.5	32.3 ± 0.5	34.0 ± 0.5	33.4 ± 0.5	31.3 ± 0.5	33.0 ± 0.5
Total (million)	6.1 ± 0.6	7.2 ± 0.5	7.4 ± 0.7	5.8 ± 0.3	6.8 ± 0.9	6.8 ± 0.3
Pregnancy rates (%)	32.2	25.7	37.5	24.4	39.6	31.7
Abortion rates (%)	16.2	14.8	11.4	27.3	5.3	14.0

Note. $p > 0.05$.

Table VIII. Total Number of Progressive Motile Sperm Inseminated

	First day				Second day				Both days		
	<2 mil	2–3 mil	3–5 mil	>5 mil	<2 mil	2–3 mil	3–5 mil	>5 mil	<3 mil	3–5 mil	>5 mil
Number of cycles	23	83	114	97	22	86	111	92	49	49	44
Pregnancy rates	13.0‡	15.6†	21.0+	41.2†,+,‡	18.2	17.4‡	23.4	33.6‡	10.2+	22.4	36.3+

Note. We only analyzed in this study data from the last 2 years; it has only been in this period that we have systematically inseminated with stimulated cycles, thus the results as function of sperm characteristics are more comparable.
$^{+}p < 0.01$; $^{\dagger}p < 0.001$; $^{\ddagger}p < 0.025$.

When only both days were considered, there was a clear difference found in the pregnancy rates: we got better results when more than 5 million total progressive sperm were inseminated in comparison with less than 3 million total progressive sperm.

DISCUSSION

Artificial insemination is the most widely used technique in assisted reproduction. Nevertheless, the use of donor sperm has decreased over the years, mainly due to the development of new assisted reproduction techniques that have permitted solutions to the most SMFs with the use of the male's own genetic material decreasing risks and generating reasonable success rates.

The introduction of intracytoplasmic sperm injection was a technological landmark; it is used in males with serious alterations in sperm production, and to a lesser degree, in cases of preimplantational diagnosis, and when dealing with sperm wash for seropositive males with HIV within a serodiscordant couple.

Nevertheless, AI is still a widely utilized option. In Spain, according to data from the Spanish Fertility Society (SEF), in 1999, more than 2500 cycles were carried out (4).

These data indicate that it is quite important for us to establish clear criteria on the candidates for these treatments, and that there is no doubt that the careful analysis of the results obtained up till now will provide us with extremely useful information.

Then, from our own results we can extrapolate the profile of the patient with better probabilities of success with AI, and also to offer an accurate prediction for each specific case.

The analysis of AI results will yield particularly valuable information regarding the whole AI technique, because of the use of donor sperm, thus normalizing the male factor that can be biasing our results in the study of infertile couples, caused by hidden abnormalities in the semen (i.e., anomalies responsible for not achieving a pregnancy by natural methods).

In general terms, pregnancy rates per cycle, per patient, and cumulative are notably higher than those previously published by diverse groups, where pregnancy rates range from 6.4 to 16.5% per cycle, at least in what is available in the international literature (5).

In the SEF register (4), the pregnancy rates of 43 Spanish centers reached 18% per cycle, thus showing a good quality level, reaching the best world results.

In the first analysis, in relation to the variety of stimulation undergone, there seems to be a generalized concordance of our results with what the literature describes, and better results are obtained with light ovarian stimulation for the development of more than one follicle, than in a NC.

Also, among the different possibilities of stimulation, use of FSH offers the best results, in comparison with the use of CC and hMG, although the latter two are still better than NC.

In other publications, the authors are not able to demonstrate any difference between stimulated cycles with CC or hMG and the natural ones; although we must remark that pregnancy rates are low (between 7% with hMG and 12.2% with CC) compared to those obtained in this work (6, 7).

Many reasons could explain this: wrong drug administration, deficiencies in the selection or preparation of the samples, a sole insemination procedure instead of the two consecutive ones (8).

We took the decision of two consecutive inseminations on the basis of the results of our own previous works, in a prospective and randomized study, that fully agrees with the available literature (9).

There are some other studies where these situations are not comparable, because there are no time coincidences or only stimulated cycles were attempted on patients that did not become pregnant in NCs (5).

Of noteworthy interest is the multicenter French work by the CECOS foundation, where in a total number of 6.083 cases, the pregnancy rate per cycle reached 8%. Also, other authors obtain pregnancy rates without ovarian stimulation in 5–11% of cases, observing that by performing subtle ovarian stimulation they can increase pregnancy rates in AI procedures. This is achieved by getting better control and prediction of the ovulation timing, together with an improvement in the production of oocytes; these are undoubtedly the reasons leading to an overall improvement in the results mainly in patients with ovarian dysfunction; the number of available oocytes is increased.

Multiple pregnancies are another important aspect of ovarian stimulation that could be considered as collateral inconvenient. Nevertheless our results clearly show that its incidence is not statistically affected by ovarian stimulation independently of the treatment considered.

The second aspect considered is the difference between the results obtained in women whose partner is affected by permanent azoospermia, or conversely by important semen abnormalities. In relation to this issue, our results clearly demonstrate that in women with an azoospermic partner, the probabilities of obtaining pregnancies are appreciably higher and this is confirmed in those situations where subtle ovarian simulation was attempted.

Nevertheless, in stimulated cycles, there is a tendency to equilibrate the results. In the bibliography, we can find works with a different methodology that reach similar conclusions (10,11).

Their reasons are that, although low, there is the possibility that males with pathological semen can obtain pregnancies in their partners naturally. In this situation, leaving aside the possibility of a third partner, the incidence of pregnancies from these pathological samples could be explained by prolonged exposure and a high female fertility.

This situation encourages us to think that in cases with high female fertility, the situation of childless couples would disappear in SMF groups which would otherwise resort to AI. Subtle stimulation would improve existing subfertility in women with SMF partner versus azoospermic male partner. Subsequently we are presenting another advantage of ovarian stimulation.

We are well aware that in a female population, there are fertile, subfertile, and infertile women depending on their probabilities of attaining pregnancy with a normal male. The difference between the groups of azoospermic males, in relation to the SMF group, is caused by the presence of spermatozoa constituting the last real possibility of achieving pregnancy with some of the fertile women.

This situation does not occur with AI in patients with azoospermia, and subsequently, the pregnancy rates for these cases must be higher, as reflected by data from Emperaire *et al.* (11), where the cumulative and pregnancy rate per cycle was 70 and 10%, respectively, for the azoospermic population in comparison with 49 and 7%, respectively, for the population with a SMF, differences being statistically significant.

In relation to the number of cycles, in their immense majority, SMFs and azoospermia are the most common circumstances where AI is recommended, while we must also bear in mind the number of women without male partners (either homo- or heterosexual), that has been increasing over recent years (data not shown). Finally, with a low number of women, we must consider genetic causes, ICSI failure, and serodiscordant couples presenting the other etiologies.

The results are very similar in all groups except for cases of ICSI failure and genetic disorders, where the rates are extremely high and approach 80% per cycle. These results are surprising, although they might be explained by the low number of cycles included.

Considering patients' age in the insemination, we clearly determined that the ages ranging between 31 and 35 yielded the highest probabilities with a notable decrease in successful outcomes from the age of 35 onwards, in both parameters of pregnancy per patient and cumulative pregnancies. A recently published work has shown that AI is a poor treatment option for women >40 years of age (12).

In any case, in women under the age of 35, amazingly, the pregnancy rates are also lower, against what was expected. Surely, the explanation can be found in the difference between the mean ages of the patients undergoing AI over a number of years (data not shown).

For women, the delay in motherhood is actually a very frequent phenomenon; a decade ago, when results were low, the mean age was also low. Over the years as we were improving our results, maternity has become a delayed stage of life for many women and hence the situation has been inverted. Furthermore, changes in the management of patients regarding ovarian stimulation have had its influence on this issue. The progressive decrease in pregnancy rates with age can be detected in unstimulated cycles, showing that stimulation seems to be relevant in relation to the results.

Another major issue is the concern that patients have in relation to the period of time that sperm has been maintained frozen. We studied the influence of the time that sperm had remained in our nitrogen banks on pregnancy rates, as well as abortion rates, for both AI and in vitro fecundation and made comparisons.

To this end, we retrospectively analyzed the above mentioned parameters as a function of time, and we can conclude that, being all groups comparable, there are no differences in either pregnancy or abortion rates, if the sample has been frozen in a period ranging from 6 months to 2.5 years.

Finally, we must consider the total number of inseminated sperm with progressive motility each day. Given the difficulties in getting good quality donors, laboratories are very interested in getting good post-thaw results as this is important in calculating statistical thresholds for successful pregnancy outcomes, i.e. the number of spermatozoa needed to obtain adequate pregnancy rates in order to optimize the use of the expensive donor sperm.

In this sense, the total motile sperm-inseminated threshold is 5 million in order to attain the desired maximum pregnancy rates. This is difficult to reach, given the difficulties in predicting the amount of frozen semen to thaw. Nevertheless, these data must be confirmed with prospective studies and must be adequately randomized before making any changes in sample management procedures. If confirmed, and should there be an increase in pregnancy rates, the mere fact that higher amounts of AI would make it a more expensive procedure and become a major economic issue.

From all the above mentioned data, to get the best results, the ideal patient profile for AI would be women under 36 years of age, without any gynecological finding, and having more than 5 million of total motile sperm inseminated in a double insemination over two consecutive days.

From the andrology lab and medical point of view, there should be a subtle ovarian stimulation with FSH and hCG, followed by insemination in order to have the highest chances of obtaining pregnancy without significantly increasing multiple pregnancy rates. On the other hand, we would discourage the practice of AI in aged patients, and mainly without ovarian stimulation, while it is essential to reach an adequate amount of sperm with good motility to inseminate. In this sense, we can guarantee the best results in patients undergoing these techniques.

AI will still be one of the most common assisted reproduction techniques in the coming years, until new methodologies developed are able to tackle each and every male fertility issue.

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