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Potential chances for natural fertility influence results of intrauterine inseminations

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ABSTRACT

Objective: Intra-uterine insemination (IUI) is widely used for different indications. The aim of the present study was to evaluate the efficiency of intra-uterine insemination as a function of indication and origin of the inseminated spermatozoa.
Study design: The retrospective study involved 827 first attempts of IUI in 827 couples between January 2011 and July 2017 in the Toulouse university hospital. Of these, 642 used fresh sperm from the husband, 40 frozen sperm from the husband and 145 frozen sperm from donors. The measured outcome parameter was live birth rate per attempt.
Results: When comparing couples lacking functional gametes (due to male or female causes), to couples who could potentially conceive spontaneously, i.e. subfertile, the latter were found to have a significantly lower live birth rate (18% vs 26%; $P < 0.05$). Even when adjusted for demographic parameters, which differed significantly between the 2 groups (female age, percentage of women suffering from primary infertility, BMI, number of inseminated motile spermatozoa and stimulation duration), this difference remained statistically significant (OR = 0.639 [0.425-0.961]; P = 0.0316).
Conclusion: When compared to couples lacking functional gametes, subfertile couples have poor IUI outcomes, suggesting a hidden cause of infertility, despite no apparent differences in ovarian reserve, tubal potency, results of ovarian stimulation and normal conventional sperm parameters. Further studies are required to better characterise and identify this subgroup of women with poor IUI outcomes.

Introduction

Intra-uterine inseminations (IUI) with fresh sperm from the husband is one of the first line treatments of subfertility. This approach can be used in cases with mild male anomaly [1,2], ovulation disorders [3] and unexplained infertility (UI) [4].

Semen cryopreservation is widely performed in cancer patients before their oncological treatments, which can affect their future fertility. The frozen spermatozoa can further be used in IUI or *in vitro* fertilization with intracytoplasmic sperm injection (IVF-ICSI) [5,6] depending on sample quality.

Ejaculation in patients with spinal cord injury can be provoked using penile vibratory stimulation or electroejaculation. The collected semen can be frozen for subsequent use in IUI [7,8].

Sperm washing and subsequent freezing can be used in HIV serodiscordant couples, where the male is infected, in order to allow his partner to conceive without the risk of contamination [9]. In these cases, washed spermatozoa, free of virus, are predominantly used in IUI (86%).

In cases of azoospermia, either intra-cervical or intra-uterine insemination with frozen donor sperm can be performed [10].

The IUI technique is well established, with some 47,323 IUIs performed in France in 2015 [11]. As results obtained in the different assisted reproduction technology (ART) centres vary extensively due to differences in indications and in treatments for ovarian stimulation [12,13], but comparisons were made between inseminations made with the same type of sperm.

In order to obtain more details about the specific impact of indications on the results of IUI, the present study aims to compare IUI outcomes as a function of the indications and the type of inseminated sperm used (fresh or frozen, husband or donor).

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Materials and methods

Patients

Eight hundred and twenty-seven couples who underwent their first IUI between January 2011 and July 2017 in a University Hospital were included in the study. The indications and the origins of the sperm used are indicated in Table 1.

Couples were excluded when the female partner did not have two potent tubes [14] or when fewer than 10^6 motile spermatozoa were obtained from the male partner after semen preparation [12] during a test performed within six months before the first IUI.

Semen preparation

Sperm was prepared according to WHO 2010 using discontinuous density gradient centrifugation (three layers: 60%, 80%, 90%) (Pure-sperm®, Nidacon, Mölndal, Sweeden). After preparation spermatozoa were incubated in 400 µl universal IVF medium (Origio, Versailles, France) at 37 °C, in a 6% CO_2 atmosphere. The number of recovered spermatozoa and their progressive motility were assessed in the medium to allow the number of recovered motile spermatozoa to be measured. Spermatozoa concentration was assessed according to Bjorndahl et al. [15] and motility according to WHO 2010.

IUI procedures

Ovarian stimulation used a combination of recombinant FSH (Gonal F, Merck, Lyon, France or Puregon, MSD, Paris, France) and GnRH antagonist (Cetrotide 0.25 mg, Merck, Lyon, France or Orgalutran, MSD, Paris, France). Ovulation was triggered with recombinant hCG (Ovitrelle, Merck, Lyon, France) when at least one follicle $\geq$ 18 mm was obtained. Insemination was performed 36 h after hCG injection. A luteal support of 400 mg per day of intra-vaginal progesterone.

Clinical pregnancy was defined as the presence of a fetal heartbeat seven weeks after insemination. Live birth was defined as the delivery of at least one live born infant after a 22 weeks or more pregnancy [16].

Statistical analysis

Data were extracted from the Gynelog clinical database used in our department. This database is approved by the French National Commission for Information Technology and Civil Liberties (CNIL) to be used for clinical research. According to French law (2012-300), patients are aware that their data can be used for anonymous clinical studies unless they specifically state otherwise. This information is detailed in posters in the rooms of the centre, and patients can inform the centre through a letter if they do not want to participate in clinical studies.

The measured primary outcome was the live birth rate per attempt.

Statistical analyses were performed using StatView software (Abacus Concepts Inc., Berkeley, CA). Data are means $\pm$ SD or median (range) according to the normality of the data. Percentages were compared by the χ^2 test. Means were compared using the Student's *t*-test and medians using the Mann-Whitney test according to the normality of data distribution.

Logistic regression included all parameters displaying a significant difference between the groups.

Results

As shown in Table 1, the live birth rate displayed large variations between indications. As some indications are rare (less than 8 couples i.e less than 1%), we decided to group indications according to the presence or absence of functional gametes for each individual couple. A lack of functional gametes could be due to the absence of female spermatozoa exposure including azoospermia, spinal cord injury (with either fresh or frozen sperm from the husband), cryopreservation before cancer treatment and HIV infected males (who had only protected sexual intercourse) or to the absence of spontaneous ovulation (amenorrhea). On the opposite side of the spectrum, some couples could spontaneously conceive as in the case of ovulation disorders with spontaneous menses, unexplained infertility, male subfertility (IUI with fresh husband sperm or with frozen sperm donor after failures of homologous IVF) and HIV infected women (after failures of intra-cervical inseminations). The latter group was called "Subfertile".

Table 2 shows that the subfertile groups had significantly lower pregnancy and live birth rate compared to the other groups but, when all couples lacking functional gametes were grouped together, this difference reached statistical significance (116/645 vs 47/182, $P < 0.05$). Because parameters such as age, BMI, AMH, stimulation length, total number of administrated FSH units, as well as the total number of motile spermatozoa inseminated, were significantly different between the 3 groups, we performed a logistic regression analysis on live birth rates, adjusting for these parameters. This logistic regression analysis also confirmed a statistically significance, approximately 40%, reduced live birth rate in subfertile couples (Table 3).

Discussion

These data show that IUI was much more efficient in the case of couples lacking functional gametes than in subfertile couples. These results are in agreement with those of Ahinko-Hakamaa et al., who reported a higher pregnancy rate in couples with anovulatory infertility (19.2%), i.e. without any chance of spontaneous pregnancy, than in cases with endometriosis (11.9%) [17]. Dinelli et al. and Cabry-Goubet et al. found similar results, between

Table 1
Results of intrauterine inseminations as the function of the origin of spermatozoa and the indications.

Type of semen used for insemination	Indications	n	N live births (%)
Fresh from husband	Amenorrhea	12	3 (25)
	Ovulation disorder	77	17 (22%)
	Unexplained infertility	463	83 (18)
	Male subfertility	77	12 (16)
	HIV infected females after failures of intra-cervical inseminations	7	0
	Spinal cord injury	6	2 (33)
Frozen from husband	Cryopreservation before cancer treatment	24	6 (25)
	HIV infected males	13	3(23)
	Spinal cord injury	3	1 (33)
Frozen from donor	Azoospermia	124	32 (26)
	IVF failure with fresh husband sperm	21	4 (19)

Table 2
Results of intrauterine inseminations as the function the couple status.

	Lack of a functional gamete			
	Absence of sperm exposure	Amenorrhea	Subfertile	Statistical comparison
n	170	12	645	
Female age	32.6 ± 4.1	31.9 ± 3.8	33.5 ± 4.0	P<0.05
Female primary infertility (%)	141 (83)	10 (83)	485 (75)	NS
Duration of infertility (m)	49 ± 30	41 ± 14.4	48 ± 22	NS
Tobacco consumption (%)				
Never smoked	88 (52)	10 (83)	374 (58)	
Current smoker	38 (22)	0	115 (18)	NS
Former smoker	44 (26)	2 (17)	156 (24)	
BMI (kg/m^2)	23.4 ± 3.7	22.7 ± 4.3	22.5 ± 3.5	P<0.05
FSH (mIU/ml)	6.9 ± 2.0	6.9 ± 2.3	7.1 ± 2.0	NS
AMH (ng/ml)	3.3 ± 2.8	11.6 ± 12.4	3.3 ± 2.9	P<0.01
AFC	23 ± 12	31 ± 10	24 ± 14	NS
Stimulation length (d)	9.9 ± 4.1	22.2 ± 9.8	9.7 ± 4.8	P<0.001
Total number of administrated FSH units	686 ± 360	1934 ± 1167	690 ± 471	P<0.001
Number of follicles ≥ 15mm	1.3 ± 0.6	1.5 ± 0.7	1.4 ± 0.6	NS
Number of inseminated motile spermatozoa (10^6)	6.8 ± 5.2	20.9 ± 15.8	20.8 ± 25.7	P<0.0001
Biochemical pregnancies (βhCG > 100UI/ml)	49 (29)	4 (33)	137 (21)	NS
Clinical pregnancies	46 (27)	3 (25)	122 (19)	NS
N fetal heart activities (%)				
1	42 (91)	3 (100)	111 (91)	
2	4 (9)	0	11 (9)	NS
≥3	0	0	0	
Live births (%)	44 (27)	3 (25)	116 (18)	NS

Table 3
Logistic regression on live birth rate after IUI (to adjust live birth rate on parameters which were significantly different between the groups).

Dependent variable: Live birth	P-value	Odds ratio	95%CI Lower	Upper
Age	0.0585	0.955	0.910	1.002
BMI	0.1076	0.956	0.904	1.010
AMH	0.9357	1.002	0.945	1.064
Lack of a functional gamete				
Yes		1		
No	0.0242	0.607	0.393	0.937
Stimulation lengh	0.2888	0.955	0.876	1.040
Total number of administrated FSH units	0.1708	1.001	1.000	1.001
Number of inseminated motile spermatozoa	0.4136	1.003	0.996	1.011

the different indications (male factor, endometriosis, mixed or unexplained infertilities), with the highest pregnancy rates obtained for ovulation disorders [3,13]. Similarly, after failure of homologous ICSI, the use of donor sperm has been reported to be more effective when the husband presented with azoospermia or cryptozoospermia [18], i.e. with no chance of spontaneous pregnancy, even after adjusting for the demographic data (OR = 1.87 [1.12-3.14]; P = 0.018).

A number of other authors however, failed to detect any differences between the various indications [19,20]. These discrepancies could be due to the different stimulation regimens used, as reported previously [12], since guidelines widely vary from one country to another [21]. For instance, IUI with donor sperm can be performed in different types of cycles, yielding significantly different clinical pregnancy rates (15% for natural cycles, 14% for clomiphene citrate cycles and 25% for gonadotropin stimulations) [22]. Also, using cryopreserved semen from cancer patients, Muller et al. [6] reported a lower live birth rate (13%) compared to our study (25%) but, since the characteristics of the patients and the methods used for IUI are not reported in their manuscript, the reasons for these discrepancies are difficult to evaluate.

Taken together, these data suggest the presence of hidden female or male anomalies, which are not exposed during conventional assessments of fertility. Indeed, numerous studies have found significant differences between UI patients and healthy fertile women during the course of non-routine investigations.

Chronic infections of the genital tract, mainly endometritis, have been found more frequently in UI than in fertile women [23,24]. Makled et al. report a diagnosis of endometritis by hysteroscopy and endometrial biopsy in 15% of UI patients [25]. Anomalous endometrial cytokine (LIF, IL17, IL18, IL35), protein (PC6) and NK cell concentrations, which may negatively impact embryo implantation, have also been reported in UI women [26–29].

The actual physiological capacity of the tubes, in the context of normal permeability, could also be raised. Indeed, we have previously reported that in cases with only one patent tube, the live birth rate after IUI was significantly reduced, suggesting a defect in the role of the tube [14]. Recently, Yucel et al. have reported that 31% of UI women had no tubal peristalsis compared to 7% of fertile women [30]. Moreover, using laparoscopy, pelvic pathologies (endometriosis, pelvic adhesions) are commonly found in IUI patients with normal hysterosalpingography [31,32].

Nutrition imbalances, concerning micronutrient as well as macronutrient intakes, have also been reported to be more frequent in IUI than in fertile patients [33].

When studying uterine haemodynamics by Doppler, an increase in the pulsatility and the resistance index has been detected in UI women when compared to fertile women [34,35].

With regards to the male, the currently available diagnostic tools are sometimes not able to explain the origin of infertility and conventional semen examination cannot always differentiate fertile and infertile men [36,37]. It is strongly suspected that damage to the

nuclear component of spermatozoa seems to play an important role in some cases of unexplained infertility where current approaches failed to identify a cause [38]. Other sperm functional tests have been proposed to explore the causes of infertility, such as computer aided sperm analysis to evaluate the motility [39], Cap-Score to assess capacitation [40], measurement of acrosome reaction [41] or human papillomavirus (HPV) sperm infection [42].

Therefore, couples with subfertility (mild male factor, low scored endometriosis or ovulation disorder without amenorrhea) could benefit from assessment identical to those of UI, since there are probably other causes of infertility, in addition to the major ones.

The main limitation of our study is that it was retrospective and observational and was performed in a single ART centre. Therefore, it must be confirmed in a large independent population.

These data, which are the first ones on the subject, should lead physicians to adapt the treatment and mainly the stimulation regimen not only to age and ovarian reserve but also to the indication, in order to optimise both the efficiency (live birth rate) and the safety (multiple pregnancy rate), and they also highlight the need for progress to further explore the causes of infertility.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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