LETTER TO THE EDITOR

Results of intrauterine inseminations with two pooled sequential ejaculates in cases of oligozoospermia

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Dear Editor,

Intrauterine insemination (IUI) is mainly indicated in cases of unexplained or mild male subfertility. In these cases, gonadotropin ovarian stimulation combined with gonadotropin-releasing hormone (GnRH) antagonist IUI leads to up to an 18% delivery rate per attempt.1 However, when the number of motile spermatozoa inseminated is lower than 1 × 10^6, the success rate drops below 5%.1 Since semen quality varies intra-individually, this threshold may not be reached on the day of IUI. In such cases, some authors have suggested asking men to provide two consecutive ejaculates and to perform IUI with the pooled spermatozoa.2 However, the effectiveness of IUI with pooled ejaculates is not clear.3

To address that question, 2162 consecutive IUI procedures performed on 869 couples at the Toulouse University Hospital, Toulouse, France, from January 1, 2012, to May 1, 2017, were analyzed retrospectively. Couples were considered eligible for IUI when at least one fallopian tube was proven permeable and the total motile sperm count (TMSC) was above 1 million after a diagnostic sperm selection test. This test was performed according to the WHO guidelines (2010)4 using discontinuous density gradient centrifugation (three layers: 60%, 80%, and 90% with PureSperm®, Nidacon, Mölndal, Sweden). Ovarian stimulations were conducted as previously described.5 For IUI, spermatozoa were selected in the same way as in the sperm selection test. If the TMSC was <1 × 10^6 after selection, a second ejaculate was requested 1 h or 2 h later. The two sperm preparations were then pooled for insemination. Clinical pregnancies were defined as the presence of a fetal heartbeat 7 weeks after insemination.

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 center, and patients can inform the center through a letter if they do not want to participate in clinical studies. Statistical analyses were performed with StatView software (Abacus Concepts Inc., Berkeley, CA, USA). Data are presented as mean ± standard deviation (s.d.). Percentages were compared with the Chi-square test and means with Student’s t-test or the Mann–Whitney U-test depending on the normality of the data distribution.

Results are presented in Table 1. Double ejaculates were necessary in 4.4% of IUI, mainly in cases of male subfertility. All basic semen parameter values were significantly lower at the time of diagnostic test selection in the cases with two sperm collections (Table 1). The threshold of 10^6 spermatozoa was obtained by pooling a second ejaculate in 82.3% (79/96) of the cases. Compared with IUI with one ejaculate (n = 2066), the clinical pregnancy rate per IUI was not lower when two ejaculates were needed (n = 96) although the number of inseminated motile spermatozoa was significantly lower. In the 17 cases where the threshold of 10^6 spermatozoa was not obtained after pooling the two ejaculates, no clinical pregnancy was obtained.

The results of this retrospective study show that compared with single-ejaculate IUI, equivalent clinical pregnancy rates are obtained when two ejaculates are pooled to compensate for an insufficient number of motile spermatozoa being available for insemination from the first ejaculate. In the early 1990s, Tur-Kaspa et al.6 showed that pooling two ejaculates increased the number of motile spermatozoa up to 329% in oligozoospermic men. Recently, Bahadur et al.7 reported that sperm concentration and progressive motility were higher in a second ejaculate collected <1 h after the first one. Barash et al.8 also reported a higher fertilization rate in in-vitro fertilization (IVF) with the second ejaculate. Based on these literature data, we hypothesize that the similar pregnancy rate in the group needing two sperm samples can be attributed to the second sperm collection. An argument for this, although in a limited number of cases, is the decreased pregnancy rate observed when the threshold of 10^6 was not reached after pooling two semen samples. In another study, Kucuk et al.9 randomized couples, whose TMSC after preparation was between 1 × 10^5 and 5 × 10^5, either with or without collection of a second ejaculate. Although the number of inseminated motile spermatozoa was significantly higher in the two-ejaculate group (7.35 × 10^5 ± 1.01 × 10^5 vs 3.99 × 10^5 ± 0.72 × 10^5; P < 0.001), the pregnancy rate did not increase significantly (6/39 vs 5/50; P = 0.444). However, the number of patients studied was too low to draw any conclusions and their thresholds for a second ejaculate (fewer than 5 × 10^6

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### Table 1: Patient characteristics, basic sperm parameters, and intrauterine insemination results based on the number of sperm collections per attempt

| Number of sperm collections per attempt | Attempt 1                  | Attempt 2                  | Statistical comparisons |
|----------------------------------------|-----------------------------|-----------------------------|-------------------------|
| Number of IU, n (%)                    | 2066 (95.6)                 | 96 (4.4)                    |                         |
| Female age (year), mean±s.d.           | 33.8±4.0                    | 32.1±4.5                    | P<0.001                 |
| Male age (year), mean±s.d.             | 36.3±3.8                    | 34.0±6.0                    | P<0.05                  |
| Infertility causes, n (%)              |                             |                             |                         |
| Ovulatory                              | 652 (31.6)                  | 20 (20.8)                   | P<0.0001                |
| Endometriosis (minimal or mild)        | 112 (5.4)                   | 1 (1.0)                     |                         |
| Male                                   | 211 (10.2)                  | 41 (42.7)                   |                         |
| Unexplained                             | 1091 (52.8)                 | 34 (35.4)                   |                         |
| Semen analysis before the IUI attempt (mean±s.d.) |                         |                             |                         |
| Volume (ml)                            | 3.7±1.5                     | 3.3±1.7                     | P<0.05                  |
| Concentration (10^6 ml⁻¹)              | 70.1±57.8                   | 36.0±32.1                   | P<0.0001                |
| Progressive motility (%)               | 37±11                       | 31±10                       | P<0.0001                |
| Vitality (%)                           | 73±12                       | 67±12                       | P<0.0001                |
| Number of spermatozoaejaculate (10⁶)   | 238.8±207.7                 | 109.5±109.4                 | P<0.0001                |
| Number of motile spermatozoa recovered after preparation (10⁶) | 20.6±24.5                  | 4.5±5.1                     | P<0.0001                |
| Number of follicles ≥15 mm at ovulation trigger (mean±s.d.) | 1.5±0.6                    | 1.5±0.6                     | NS                      |
| Number of motile spermatozoa inseminated (10⁶), mean±s.d. | 21.9±25.0                  | 3.3±3.3                     | P<0.0001                |
| Clinical pregnancies, n (%)            | 324 (15.7)                  | 13 (13.5)                   | NS                      |

IU: intrauterine insemination; s.d.: standard deviation; NS: not significant

### Author Contributions

JM participated in the design of the study, analysis of the results, carrying out IU, and writing the manuscript. NG participated in carrying out IU and writing the manuscript. JP contributed to the design of the study, statistical analyses, carrying out IU, and writing the manuscript. RL contributed to carrying out IU and writing the manuscript. All authors read and approved the final manuscript.

### Competing Interests

All authors declare no competing interests.

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