TITLE: - Reproductive Outcomes in Lesbian Couples Undergoing Reception of Oocytes from Partner Versus Autologous In Vitro Fertilization/Intracytoplasmic Sperm Injection

RUNNING TITLE: ROPA vs. IVF/ICSI in lesbian couples

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EXTENDED ABSTRACT:

Study question: Is live birth after Reception of Oocytes from Partner (ROPA) comparable to classic *in vitro* fertilization-intracytoplasmic sperm injection (IVF/ICSI) in lesbian couples?

Summary answer: The ROPA technique presents a higher live birth rate compared to classic IVF/ICSI in the studied population.

What is known already: While in classic IVF/ICSI a woman is, at the same time, the provider of the oocytes and the recipient of the embryos, in ROPA the process is shared between the partners: one of them undergoes ovarian stimulation and ovum pick-up, whilst the other undergoes endometrial preparation and carries the pregnancy. Although ROPA is increasing popular among lesbian couples, no clear understanding of its outcomes is present in the literature, making it difficult for clinicians to properly counsel these couples.

Study design, size, duration: Retrospective matched cohort study of lesbian couples in a large fertility center having performed a cycle between February 2012 and May 2018. The study included 210 couples: 70 that underwent for the first time ROPA and 140 that underwent, also for the first time, classic IVF/ICSI.

Participants/materials, setting, methods: ROPA and IVF/ICSI couples were matched 1:2 by age of the woman providing the oocytes (±5 years), day of ET (D+2 or D+5) and number of transferred embryos (1, 2 or 3). Laboratory and clinical outcomes were compared between groups using univariable (Pearson's Chi^2 test) and multivariable analyses (logistic regression) adjusted for age of the woman providing the oocytes and BMI of the woman receiving the embryos.

Main results and role of chance: Ovarian stimulation led to 9.1 (SD 4.5) mature oocytes (MII) in ROPA vs. 8.2 (SD 4.5) in IVF/ICSI (p=0.16). Fertilization rate was 73.6% in ROPA vs. 76.2% in IVF/ICSI (p=0.37). Clinical outcomes in ROPA vs. IVF/ICSI were: biochemical pregnancy rate 68.6% vs. 46.4% (p=0.002); clinical pregnancy rate 57.1% vs. 38.6% (p=0.011), ongoing pregnancy rate 55.7% vs. 35.7% (p=0.006), and live birth rate 53% vs. 29.3% (p=0.001). After adjusting for age and BMI, we still observe a significant improvement in ROPA for biochemical pregnancy (OR=2.1, 95%CI 1.10, 4.03; p=0.025), clinical pregnancy (OR=2.11, 95%CI 1.10, 4.07;

p=0.025), ongoing pregnancy (OR=2.29, 95%CI 1.18, 4.42; p=0.014), and live birth rates (OR=2.68, 95%CI 1.37, 5.26; p=0.004). Our results suggest that ROPA might be more efficient than classical IVF/ICSI in selected lesbian couples.

Limitations, reasons for caution: It has to be considered that: 1) oocytes' age was significantly lower in ROPA (better prognosis); 2) More IVF/ICSI patients going into this treatment after previous failed intrauterine insemination (IUI) treatments (worse prognosis), and 3) ROPA recipients underwent endometrial preparation but not ovarian stimulation (better uterine conditions).

Wider implications of the findings: ROPA allows improved treatment participation for lesbian couples, and it might improve reproductive outcomes through the possibility of selecting the best combination between two oocyte providers and two gestational mothers. As oocyte donation pregnancies present higher hypertensive disorders, a careful evaluation of risks and benefits is recommended before advising this treatment.

Study funding/competing interest(s): None

KEYWORDS: ROPA; IVF; ICSI; Co-IVF donor insemination; ART; oocyte donation; ageing; SSFCs.

INTRODUCTION

Historically, the concept of family only contemplated heterosexual married couples forming a traditional family structure. Nevertheless, over the last several decades, social acceptance has widened and our society has expanded the concept of family in order to comprise not only this group but also unmarried couples, single parents, and lesbian, gay, bisexual and transgender (LGBT) couples. Alongside to this change of concept, LGBT couples have been using assisted reproductive technologies (ART) and the perception of parenthood has experienced a wider transformation.

Same-sex female couples (SSFCs) in Spain have had legal access to the utilization of donor insemination (DI) since the first sperm bank was launched in 1978. More recently, the national Spanish legislation allowed matrimony between homosexual couples and equalized their reproductive rights with those of heterosexual couples (Law, 2005). Until this law was adopted in Spain, the female partner of the woman treated with DI had no legal rights towards the child. The only option that could be appealed was to undergo DI both women using the same donor. In one hand, these women had a common reproductive project as in case of giving birth each women in a lesbian couple to children on their own, among them they would become half-siblings. On the other hand, they shared no biological maternity. The current legislation makes it possible for both women in a lesbian couple to be parents using ART. It is worth to mention that assisted reproductive technologies (ART) in Spain were first regulated in 1988 (Law, 1988), and updated in 2006 (Law, 2006).

The utilization of ART, particularly by SSFCs, has increased and gone through a process of improvement and evolution over the past decades. Women of SSFCs typically have elected to undergo DI or IVF/ICSI according to multiple factors such as gynecological history or the age of the women before pursuing any ART. Co-in vitro fertilization (Co-IVF), also known as Reception of Oocytes from Partner (ROPA), is a reproductive medical intervention in which one partner provides her oocytes, after hormonal stimulation and oocyte retrieval, which will be fertilized with donor sperm to generate the embryos that will be afterwards placed in the uterus of the partner, who will carry on with the pregnancy and the delivery. Technically, the ROPA process does not differ from an oocyte donation process although it does substantially change as it

takes place between partners and SSFCs will always require a donor sperm. The term "partner donation" first came into the field through the (Directive2004/23/EC) and the term "donor" in the ART context is assigned to a third party who provides gametes or embryos who is not participating in the parental project (Pennings, 2016). According to this definition, ROPA is not a donation since the woman who provides the oocytes intends to use them for her own reproduction, which makes a big difference at a human level.

These cases of ART turn up different conceptions of parenthood: genetic parenthood, where parenthood is understood as arising from genetic derivation; gestational parenthood, where parenthood arises from pregnancy and childbirth; and intentional parenthood, where parenthood arises from the intention to bring into existence and/or rear the child. The term biological parenthood commonly refers to genetic and/or gestational parenthood (Zeiler and Malmquist, 2014). As co-IVF is a relatively novel strategy for SSFCs seeking a shared experience where both women physically contribute to the pregnancy, few reports about this new fertility strategy have been published yet. The first published European study reported the pioneering experience of a Spanish group from Barcelona (Marina, et al., 2010) on 14 same-sex couples. A more recent study reported a similar positive experience from a single centre in New York between 2002 and 2014 (Yeshua, et al., 2015). To date, the largest published series so far is a 6-year retrospective study from a single, private centre in United Kingdom, which included 121 consecutive lesbian couples undergoing ROPA treatment. Yet, no article has ever been published comparing this new method with the traditional one still offered since its inception (IVF/ICSI), which is the aim of the present study.

MATERIALS AND METHODS

Study population

This is a retrospective matched cohort study of lesbian couples in a large private fertility center. The study included 210 couples: 70 ROPA couples matched (1:2) with 140 couples that underwent classic IVF/ICSI, patients in both groups undergoing treatment for the first time. ROPA and IVF couples were matched by age of the woman providing the oocytes (±5 years), number of transferred embryos (1, 2 or 3), day of the ET (day 3

or 5 of embryo development), and fresh or frozen embryo transfer (ET). We analyzed the results of 210 ETs performed between February 2012 and May 2018.

Medical protocol

Women pursuing IVF followed ovarian stimulation with exogenous FSH (Gonal®, Merck Serono, Spain) or purified human menotrophin (Menopur® FERRING GmbH, Germany) in doses of 150-300 IU/day on the second day of the cycle, on a GnRH antagonist protocol (Cetrotide®, Merck Serono, Spain), 0.25mg/day fixedly from the sixth day of stimulation and triggered with 250 µg of hCG (Ovitrelle®, Merck, Germany). Women providing the oocytes for ROPA followed the same ovarian stimulation, but triggering ovulation with 0.3 mg of the GnRH agonist Triptorelin (Decapeptyl® Ipsen Pharma Biotech, France). In addition, women undergoing the ET for ROPA underwent endometrial preparation with estrogens, administered either orally (Progynova, Bayer Hispania S.L., Spain; 6 mg/day) or transdermally (Estradot Novartis Pharma GmbH, Germany; 150µg/day). In both cases, IVF and ROPA, ovulation was triggered when 3 or more follicles ≥17 mm of diameter were present on the ovaries, and OPU was performed 36 hours after triggering, by means of ultrasound guided transvaginal follicular aspiration. In both cases too for women who underwent the ET, luteal phase was supported with vaginal progesterone 400mg/12h (Utrogestan®, SEID SA, Spain or Progeffik®, Effik, Spain) from OPU until 14 days after ET, and continued in case of a positive beta-hCG test until week 12 of pregnancy.

Statistical analysis

We compared pregnancy outcomes between ROPA and IVF/ICSI couples using univariable analyses (Pearson's Chi^2 test), and multivariable analyses (logistic regression) adjusted for age of the woman providing the oocytes and BMI of the recipient. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS software, version 22.0.

Ethical approval

Approval from the institutional Ethics Committee for Clinical Research was obtained before the implementation of this study.

RESULTS

Age and BMI characteristics of all women included in the study are reported in Table I. Regarding cycle characteristics, we observe that couples undergoing IVF/ICSI had underwent previous IUI treatment 2.7 more times in comparison to ROPA couples: 92 (65.7%) vs 17 (24.3%), ($p<0.001$). It is also noticeable that most ETs in both groups of women were performed on D2-D3 of embryo development (86.2%) with transfer of 2 embryos (80%). Overall, 180 ETs were performed in fresh while 30 were elective frozen ETs.

In relation to laboratory outcomes, ROPA led to 9.1 (SD 4.5) mature oocytes (MII) vs. 8.2 (4.5) in IVF/ICSI ($p=0.16$). No significant differences were observed when it comes to fertilization rate between ROPA and IVF/ICSI (73.6% vs 76.2%, $p=0.37$). Reproductive outcomes were significantly better in the ROPA group compared to the IVF/ICSI group: biochemical pregnancy rate was 68.6% vs. 46.4% ($p=0.002$); clinical pregnancy rate 57.1% vs. 38.6% ($p=0.011$), ongoing pregnancy rate 55.7% vs. 35.7% ($p=0.006$), and live birth rate 53% vs. 29.3% ($p=0.001$). After adjusting for age and BMI in the multivariable analysis, we still observe a significant improvement in the ROPA group for all the clinical outcomes: biochemical pregnancy (OR 2.10, 95%CI 1.10, 4.03; $p=0.025$), clinical pregnancy (OR 2.11, 95%CI 1.10, 4.07; $p=0.025$), ongoing pregnancy (OR 2.29, 95%CI 1.18, 4.42; $p=0.014$) and live birth (OR 2.68, 95%CI 1.37, 5.26; $p=0.004$).

DISCUSSION

This is the first published study to compare live birth rates between ROPA and classic IVF/ICSI in lesbian couples, and showing a higher live birth rate in those undergoing ROPA (23.8% more than live birth compared to IVF/ICSI).

ROPA is an increasingly requested choice of ART that offers improved treatment participation for lesbian couples. In addition, it permits a woman who has a functional uterus but no oocytes or insufficient quality of oocytes to experience pregnancy and become a gestational mother to a child who has a genetic bond to her partner. Similarly, it allows a woman who has good quality oocytes but no functional uterus to become the genetic mother of a child carried by her partner.

Previous published studies only provided descriptive statistics of the ROPA cycles performance, without a comparison with IVF/ICSI. The Finnish study from Yeshua et al. (Yeshua, Lee, Witkin and Copperman, 2015) reported 141 cycles of traditional IVF from a total of 177 cycles (the other 36 cycles being ROPA), but did not compare results between the two techniques. When comparing reproductive results of ROPA in our study and in the previous ones, we observe that our live birth rate after the first embryo transfer is significantly higher (53%) that that reported in the first ROPA study performed in Europe (7.7%) (Marina, Marina, Marina, Fosas, Galiana and Jove, 2010) and in the study from Yeshua et al (25%) (Yeshua, Lee, Witkin and Copperman, 2015). However both studies included a few number of cycles (13 and 36 cycles, respectively). In addition, the study of Yeshua et al., did not publish live birth results for all the cycles as 5 cycles were still ongoing when the study was published, but still the biochemical pregnancy rate from their study was considerably lower than ours: 13.8% vs 68.6% ($p=0.002$). The largest study published until now, which included 121 couples undergoing ROPA (Bodri, et al., 2018), reported a cumulative live birth rate of 60%, unfortunately we do not have cumulative results and they do not report first ET results which makes it challenging to compare the results of both studies.

A reason that could explain our better results for the ROPA technique is the age of the participants, specially the oocyte provider's. In our study 54.3% of women providing the oocytes were younger than 35 years old, mean age of women providing the oocytes in the ROPA group being 34.0 years. In the study of Yeshua et al. this age group of women represented a 41.7%, whereas in the study of Marina et al. the mean age of oocyte providers was 35.1 years. Focusing on our study, despite couples were matched by age, the oocytes' age was significantly lower in ROPA than in IVF/ICSI because age±5 years was allowed, due to the difficulty of finding a perfect match with IVF/ICSI couples. Another reason for better results in the ROPA group is that women receiving the embryos had to undergo endometrial preparation but not ovarian stimulation, which confers better uterine conditions for these women comparing to those undergoing IVF/ICSI. In addition, significantly more IVF/ICSI couples went through this treatment after previous failed IUIs, which could account for a worse prognosis compared to the ROPA group.

Focusing on the reproductive results of the IVF/ICSI group in our study (29.3%), we observe that they are slightly worse than those reported in two previous studies using this technique in the same population. Nordqvistet et al. (Nordqvist, et al., 2014) and Carpinello et al. (Carpinello, et al., 2016) reported a live birth rate of 38% and 46.9%, respectively. Conversely, the clinical pregnancy rate in our study (38.6%) was moderately superior to that reported by Fiske et al. (34.4%) (Fiske and Weston, 2014). Nevertheless, all these results are still lower than those obtained for the ROPA group in our study.

We have to recognize some limitations of our study. First, the cohort of patients included this study may not be representative of all the lesbian couples accessing ART. This is because, although ROPA offers some advantages in comparison to classical techniques (IVF/ICSI and IUI), it is not a technique applicable to all lesbian couples who seek to create a family. First of all, they must meet specific legal conditions; in Spain, the ROPA technique is not specifically regulated, but lesbian couples have to be married to go through it for the recognition of both women as parties of the couple treatment and parents of the newborn. Once this is solved, they need to gather medical conditions in order to be offered the treatment. As we have previously mentioned, ROPA does not technically differ from an oocyte donation (double donation) cycle, and it is known that the use of donated gametes is an important risk factor for preeclampsia (Blazquez, et al., 2018). This risk should be evaluated when the technique is offered to couples, who should be further monitored. Though, adverse events occurred during pregnancy and/or perinatally were not the within the extent of this study.

In conclusion, the results presented in this study suggest that ROPA might be more efficient than classical IVF/ICSI in eligible lesbian couples. These data can be used to better counsel these couples regarding expectations of their fertility treatment. At the end, regardless of gametes source, fertility centers have to make their best to maximize the chance that a healthy baby is born, minimizing the risks.

AUTHORS ROLES

AN: involved in study design, data analysis and manuscript preparation. D.G: involved in study design, statistical analysis and manuscript preparation. P.G.B: involved in study

design and manuscript preparation. R.V. and A.R.: involved in study design, expert knowledge, manuscript preparation.

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CONFLICT OF INTEREST

The authors have nothing to declare.

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Table I. Demographic characteristics overall and by study group.

	ROPA
Age of Woman 1, Mean (SD)	34.3 (5.8)[1]
Age of Woman 2, Mean (SD)	34.0 (4.5)
BMI of Woman 1, Mean (SD)	24.2 (4.5)
BMI of Woman 2, Mean (SD)	24.0 (4.3)[2]

	IVF
Age of Woman 1, Mean (SD)	34.2 (3.9)
Age of Woman 2, Mean (SD)	36.7 (7.0)
BMI of Woman 1, Mean (SD)	23.2 (3.8)[2]
BMI of Woman 2, Mean (SD)	23.8 (3.9)

[1]Age of the oocyte provider in ROPA and IVF

[2]BMI of the oocyte receiver in ROPA and provider in IVF