Impact of Oocyte Retrieval Number on Cumulative Live Birth Rates in ICSI Cycles: A Retrospective Study

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Abstract: *Aim: To study the association between the number of oocytes retrieved and cumulative live birth rates (CLBR) Design: Retrospective analysis of patients who underwent autologous first ICSI cycle. Setting: Institute of Reproductive Medicine, Madras Medical Mission Hospital, Chennai. Methods: Patients undergoing first ICSI cycle from January 2017 to December 2019. The study included 275 patients who were grouped based on the number of oocytes retrieved (<5, 6 - 10, 11 - 15, 16 - 20, 21 - 25, 26 - 30, >30). They were further sub grouped into categories based on their age, BMI and AMH. The cumulative LBR was calculated on per - retrieval basis and was defined as at least 1 live (more than 28 weeks) birth from all linked embryo frozen transfers. Result: The cumulative LBR increased rapidly with the number of oocytes retrieved up to 10, plateaued from 11 - 15 oocytes, after which there were diminishing returns. Conclusion: GnRH Antagonist stimulation with 10 - 15 oocytes had a cumulative LBR of 66.7% in the first IVF cycle with frozen embryo transfers. Beyond 15 oocytes, the CLBR declined.*

Keywords: Oocyte retrieval, Cumulative Live Birth Rate, ICSI, IVF, Reproductive medicine

1. Introduction

Numerous studies have investigated the relationship between the number of oocytes retrieved during ovarian stimulation and live birth outcomes. When considering the cumulative LBR, studies have generally reported a positive trend where more oocytes result in a higher cumulative LBR, with no apparent detriment to retrieving a high number of oocytes [1]. Data are conflicting regarding the effect of gonadotropin dose and high oocyte yield on aneuploidy rates, with some studies noting a dose - related effect and others refuting this association [2].

The efficacy of ICSI cycles hinges on various factors, with the number of oocytes retrieved emerging as a crucial consideration. Despite advancements in ART techniques, there persists an ambiguity surrounding the precise association between the quantity of oocytes retrieved during an ICSI cycle and the subsequent cumulative live birth rates [3].

The number of oocytes retrieved is commonly perceived as a critical determinant of treatment outcomes in ICSI cycles. A higher number of retrieved oocytes may offer a wider selection for fertilization, potentially increasing the chances of successful embryonic development and implantation. Conversely, a lower number of retrieved oocytes may limit the available options, potentially impacting treatment success rates.

Registry analyses of significant scale consistently indicate that live birth rates (LBRs) reach a plateau when the count of harvested oocytes falls within the range of 10–15, with a further escalation in oocyte count posing an elevated risk of ovarian hyperstimulation syndrome (OHSS) [4], [5]. But these analyses solely focus on the outcomes of only fresh IVF cycles, neglecting to consider the potential advantages associated with the transfer of surplus frozen embryos in subsequent cycles, thereby not accounting for the cumulative LBR after utilizing all fresh and frozen - thawed embryos following a single ovarian stimulation protocol.

We gathered data spanning from 2017 to 2019 at the Institute of Reproductive Medicine, Madras Medical Mission Hospital. This data encompasses all oocyte retrievals (first IVF cycle) and their corresponding cumulative live births.

2. Materials and Methods

This is a retrospective study conducted in the Institute of Reproductive Medicine, Madras Medical Mission Hospital from January 2017 to December 2019. The study was approved by the Institutional Scientific and Ethical Review Boards.

All women between 25 and 45 years old undergoing their first IVF ovarian stimulation cycle, in a GnRH antagonist protocol, undergoing frozen embryo transfers using cleavage /morula stage embryos only were considered as inclusion criteria. Exclusion criteria were genetic diagnosis or screening, oocyte donation, or having adenomyosis, endometriosis, uterine anomalies, fresh embryo transfers, no embryo transfer after retrieval, or high DFI in males.

Treatment Protocol:
Patients were administered daily injections of gonadotropins, commencing on day 2/3 of their menstrual cycle. Monitoring of the cycle included assessments of serum E2, P, and LH

levels, along with serial transvaginal ultrasounds. The introduction of the GnRH antagonist was done on day 6 of stimulation, or when the leading follicle reached a mean diameter of 14 mm. Ovulation was triggered using human chorionic gonadotropin, or GnRH agonist.

The collected oocytes were inseminated using ICSI. Embryos were cultured up to day 2 or day 3 after oocyte retrieval and were vitrified using an open vitrification system. Embryo transfer of only cleavage/morula frozen - thawed embryos was done through artificial preparation, with or without GnRH down - regulation. Embryo transfers were done when the endometrial thickness was 8 mm and above.

LBR Calculation
The final outcome of this study is to find out if patients included in the study had at least one live (more than 28 weeks) birth from all linked frozen embryo transfers, irrespective of the interval between retrieval and transfer, encompassing the cleavage - stage transfers only. In our study, we analyzed a cohort of 275 patients (N = 275), who were then divided into groups based on whether they had a live birth or not. Subsequently, they were further categorized according to the number of oocytes retrieved.: Group 1 (<5) (n = 51), Group 2 (6 - 10) (n = 102), Group 3 (11 - 15) (n = 76), Group 4 (16 - 20) (n = 30), Group 5 (21 - 25) (n = 11), Group 6 (26 - 30) (n = 3) and Group 7 (>30) (n = 2). A Chi square test was done to compare the association between age, BMI, AMH, the number of oocytes retrieved, and the cumulative live birth rate. If the P value was < 0.05, it was considered significant.

3. Results

In our study, a total of 275 (N) patients were analyzed, of whom 117 patients had live births combining all linked embryo transfers. The number of oocytes retrieved on average was 10 (Interquartile range, 5 - 17) and the maternal age was an average of 32 years (Interquartile range, 28 - 36). The cumulative live birth rate was found to be in increasing trends until retrieval of 10 oocytes, after which it plateued up to 15 oocytes and then it started to decline.

In Table 1, basic variables compared with the oocyte - stratified group. When comparing patient age and the number of oocytes retrieved, the highest number of oocytes was observed in patients under 30 and up to 35 years old. Additionally, as age increased, there was a notable decrease in the number of oocytes retrieved. Specifically, groups 2, 3, and 4 consisted of more patients under 30 and up to 35 years old, while groups 1 and 2 had a higher proportion of patients aged 36 years and above.

The maximum number of patients were in the BMI range of 18.5 - 24.9 (n = 168), but there was no statistical difference between the BMI and the number of oocytes retrieved. On comparing the number of oocytes and AMH, groups 2, 3, 4 had maximum patients with AMH levels ranging from 2.1 to 3.9 ng/mL. Conversely, group 1 had a greater proportion of patients with AMH levels below 1 ng/ml, whereas patients with AMH levels between 1 and 2 ng/ml were predominantly found in groups 2 and 3. Thus, with decreasing AMH, there is a significant decrease in the number of oocytes retrieved.

The basic characteristics of patients with or without live birth are listed in Table 2. When comparing the fundamental characteristics between those with and without live births, no significant differences were observed in terms of patient age, BMI, AMH levels, or the cause of infertility. But there was decrease in the number of oocytes retrieved with increasing age and decreasing AMH which were statistically significant (Table 1). Our analysis showed that the cumulative live births were higher between 10 - 15 oocytes retrieved as shown in Figure 1 with a cumulative live birth rate of 66.7%.

Table 1: Basic variables compared with the Oocyte - stratified group

Variables (N=275)	Group 1 (<5) (n=51)	Group 2 (6 - 10) (n=102)	Group3 (11 - 15) (n=76)	Group 4 (16 - 20) (n=30)	Group 5 (21 - 25) (n=11)	Group 6 (26 - 30) (n=3)	Group 7 (>30) (n=2)	P value
Age								
<30yrs (94)	7.4% (7)	34.0% (32)	37.2% (35)	16.0% (15)	4.3% (4)	1.1% (1)	0.00%	<0.05
31 - 35yrs (103)	15.5% (16)	35.9% (37)	28.2% (29)	12.6% (13)	4.9% (5)	1.0% (1)	1.9% (2)	NS
36 - 40yrs (65)	33.8% (22)	43.1% (28)	16.9%11	16.9% (1)	3.1% (2)	1.5% (1)	0.00%	NS
>41yrs (13)	53.8% (7)	30.8% (4)	7.7% (1)	7.7% (1)	0.00%	0.00%	0.00%	NS
BMI								
<18.5 (0)	0%	0%	0%	0%	0%	0%	0%	NS
18.5 - 24.9 (168)	16.1% (27)	36.9% (62)	28.6% (48)	13.1% (6)	3.6% (6)	0.6% (1)	1.2% (2)	NS
25 - 29.9 (97)	22.7% (22)	35.1% (34)	27.8% (27)	8.2% ()	4.1% (4)	2.1% (2)	0%	NS
>30 (10)	30% (3)	50% (5)	10% (1)	0%	10% (1)	0%	0%	NS
AMH								
<1.0 (34)	73.5% (25)	11.8% (4)	11.8% (4)	2.9% (1)	0%	0%	0%	NS
1.0- 2.0 (56)	14.3% (8)	58.9% (33)	23.3% (13)	3.6% (2)	0%	0%	0%	NS
2.1 - 3.9 (136)	12.5% (17)	41.9% (57)	32.4% (44)	9.6% (13)	2.9% (4)	0.7% (1)	0%	<0.05
>4.0 (49)	4.1% (2)	14.3% (7)	30.6% (15)	28.6% (14)	14.3% (7)	4.1% (2)	0%	NS

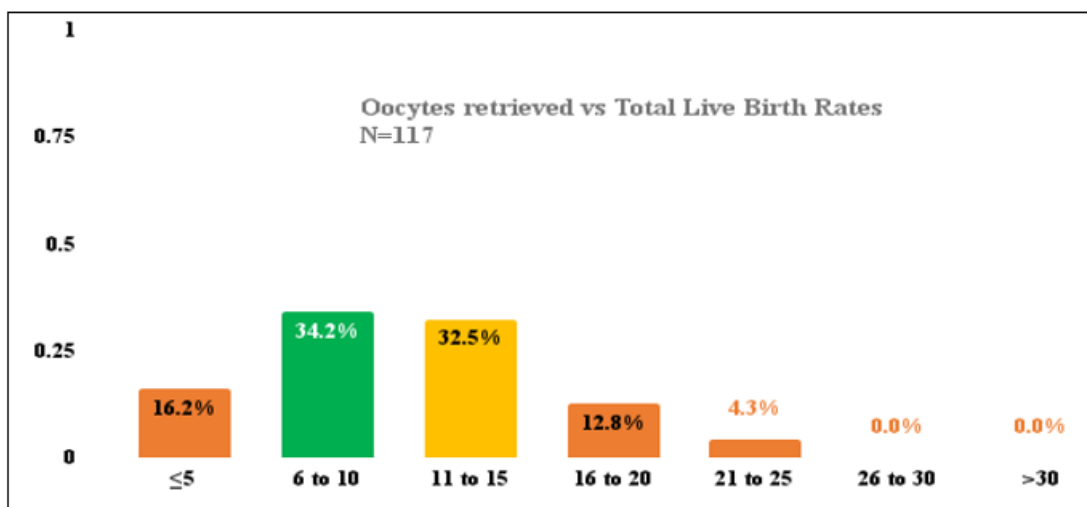| No of oocytes	total live birth rates
≤5	19 (16.2%)
6 to 10	40 (34.2%)
11 to 15	38 (32.5%)
16 to 20	15 (12.8%)
21 to 25	5 (4.3%)
26 to 30	Nil
>30	Nil
Total	117

Figure 1: Comparison between oocytes retrieved and Cumulative live birth rates

Table 2: Basic characteristics of the patient and their treatment outcomes

Variables	Live Births (N=117)	No Live Birth (N=158)
Age		
<30yrs (94)	47 (40.2%)	47 (29.7%)
31 - 35yrs (103)	47 (40.2%)	56 (35.4%)
36 - 40yrs (64)	21 (17.9%)	44 (27.8%)
>41yrs (13)	2 (2%)	11 (7.1%)
BMI		
<18.5 ()	0	0
18.5 - 24.9 (168)	70 (59.8%)	98 (62.1%)
25 - 29.9 (97)	42 (35.9%)	55 (34.8%)
>30 (10)	5 (4.3%)	5 (3.1%)
AMH		
<1.0 (34)	12 (10.3%)	22 (13.9%)
1.0- 2.0 (56)	20 (17.1%)	36 (22.8%)
2.1 - 3.9 (136)	60 (51.3%)	76 (48.1%)
>4.0 (49)	25 (21.4%)	24 (15.1%)
Cause of infertility		
Male factor (62)	22 (18.8%)	40 (25.3%)
POR (47)	16 (13.7%)	31 (10.1%)
PCOD (38)	16 (13.7%)	22 (13.9%)
Tubal (27)	10 (8.5%)	17 (10.7%)
Unexplained (101)	53 (45.3%)	48 (30.3%)

4. Discussion

Our study results indicate a comparable association with the two previous registry analyses done using Human Fertilization and Embryology Authority (HFEA) national database by Smith et al. [6] and McLernon et al. [7], in which cumulative LBR reached a plateau beyond 15 and 13 oocytes, respectively. But in these analyses, cumulative live - birth rates across repeated stimulation cycles were compared, in contrast to our study, where only the first IVF cycle was considered as the study population. Our study further differs from these analyses in the type of stimulation protocols utilized, as we used only the GnRH antagonist protocol with GnRH agonist triggering, while these analyses included many long acting GnRH agoint protocols.

Various single - center studies [1], [8], [9], and [15], demonstrate an increase in cumulative LBR with an increasing number of oocytes retrieved. The reason behind this maybe that higher oocyte yield categories might generate surplus embryos, thus potentially enhancing the overall cumulative LBR. However, an increased number of oocytes may only contribute to an increase in the risk of ovarian hyperstimulation syndrome (OHSS). Further, two registry analyses done by Steward et al. and Sunkara et al. [4], [5] show that LBRs plateau when 10–15 oocytes are collected, and additional oocytes may only heighten the risk of ovarian hyperstimulation syndrome (OHSS) without further improving outcomes. Magnusson et al. [13] proposed that obtaining 18 oocytes would be ideal for achieving cumulative live births across all age groups. Chen et al. [14] from China noted that for patients with PCOS, the Cumulative Live Birth Rate did not increase when the number of oocytes exceeded 10.

In the other subgroups that we compared in our study, the number of oocytes retrieved were higher in younger age group with optimal AMH (2.1 - 3.9 ng/mL), but BMI did not show any statistical significance for the quantity of harvested oocytes. Two large registry studies [10] and [11] also manifest the significance of age affecting the quantity of oocytes collected. Despite considerable heterogeneity across parameters including sample size, patient age, study design, stimulation protocols, and more among the various studies we reviewed, they collectively demonstrated a robust positive

correlation between the quantity of retrieved oocytes and the Cumulative Live Birth Rate (CLBR) in conventional in vitro fertilization approaches.

The limitation of our study is that it is retrospective, and we tried to minimize selection bias by having strict inclusion criteria and by controlling various confounding factors. Our study does not include fresh transfers, and only frozen cleavage/ morula stage embryo transfers were analyzed. But even in the previous studies mentioned earlier [4] and [5], the LBR decreased with an increasing number of oocytes due to the detrimental effect of excessive ovarian response on endometrial receptivity and embryo implantation rates. But the data on the number of embryos used, endometrial preparation method, endometrial thickness, and progestrone level on the day of transfer were not analyzed in our study.

Many studies have demonstrated different optimal oocyte quantities for optimal CLBR. Even after the above drawbacks, our study holds good for the current clinical practice of having OHSS - free cycles with GnRH antagonist stimulation and freeze - all strategy. An optimum of 10 - 16 oocyctes retrieved will be an efficient and safe approach. Also, two large scale studies on mild stimulation [11] and [12] propose that acquiring a reduced number of oocytes may result in a decreased embryonic aneuploidy rate and an enhanced implantation rate.

In conclusion, this study found that the CLBR increased up to 10 oocytes, plateaued from 11 - 15 oocytes, and started to decline after that in all age groups. The number of oocytes retrieved was higher in the younger age group with normal AMH. GnRH antagonist cycles with 10 - 15 oocyte retrieval had a cumulative LBR of 66.7% in the first IVF cycle with frozen embryo transfers. Further studies to investigate the euploid nature of the embryos and this low oocyte yield can be done in the future.

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