The total pregnancy potential per oocyte aspiration after assisted reproduction—in how many cycles are biologically competent oocytes available?

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Abstract

Purpose While stimulation of women prior to assisted reproduction is associated with increased success rates, the total biological pregnancy potential per stimulation cycle is rarely assessed.

Methods Retrospective sequential cohort study of the cumulative live birth rate in 1148 first IVF/ICSI-cycles and 5-year follow up of frozen embryo replacement (FER) cycles were used. Oocyte number, number of embryos transferred, and cryopreserved/thawed and transferred embryos in a FER cycle were registered for all patients. Children per oocyte and per transferred embryo and percentage of cycles with births were calculated.

Results We obtained 9529 oocytes. Embryos (2507) were transferred in either fresh or FER cycles, resulting in 422 births and 474 live born children. Median age of the women was 32.5 years (range 20–41.5 years). In total, 34.3 % of all cycles ended with a live birth while in 65.7 % of the cycles, no oocytes were capable of developing into a child. The average number of oocytes needed per live born child after transfer of fresh and thawed embryos was 20 as only 5.0 % of oocytes aspirated in the first IVF/ICSI cycle had the competence to develop into a child.

Conclusions In our setting, overall 5.0 % of the oocytes in a first cycle were biologically competent and in around 2/3 of all

cycles, none of the oocytes had the potential to result in the birth of a child.

Keywords In vitro fertilization · Competence · Oocytes · Utilization · Live birth · Assisted reproduction

Introduction

Both ongoing pregnancy and live birth per cycle/per embryo transfer are commonly used to measure the success of assisted reproduction technology (ART) treatments. However, cumulative live birth rate (cLBR) per cycle or oocyte to baby rates is a better measure of the biological potential of a cohort of oocytes.

Several different birth rates per cycle have been published. This includes the number of live births (after transfer of fresh embryos) per oocyte retrieved (live birth rate) [1] and the (calculated) cumulative number of live births per oocyte retrieved, where the calculated live birth from cryopreserved embryos was added to the live birth rate (LBR) in the fresh cycle [2, 3]. Other suggestions have been “oocyte to baby rate” [2] defined as the number of live births from all the collected oocytes. Sometimes, one term is used for different contexts. This includes “oocyte utilization rate” which has been used to describe both “the rate of live baby born per oocyte” (LBB/oocyte) and number of oocytes resulting in embryos with a quality compatible with cryopreservation or fresh transfer.

Only one previously published study reports the cumulative LBR (cLBR) per cycle including the outcome of both the fresh and subsequent cryopreservation cycles [4]. Other studies have focused on number of live births per mature oocyte in the fresh cycles [5] or after oocyte cryopreservation (OC) per thawing cycle [6–8]. All these calculations are useful not only to elucidate the overall efficiency/inefficiency of an ART cycle but also because it provides more detailed information

Capsule In our setting, overall 5.0 % of the oocytes in a first cycle were biologically competent and in around 2/3 of all cycles, none of the oocytes had the potential to result in the birth of a child.

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concerning competence of the gametes and embryos and thereby offering more realistic expectations for a given cycle [9]. Several studies have shown that it may be preferential to go for minimal stimulation as high oocyte numbers do not necessarily increase the number of babies per oocyte cohort [2, 3, 8]. In addition, a good estimation of the reproductive efficiency of the oocytes retrieved is important in order to provide the patients with realistic estimations of a successful outcome of their treatments. Further, it offers more equal means by which to compare technologies, thereby improving the quality of counseling for both patients and practitioners.

Often the failure to establish a pregnancy during ART treatment is attributed to wrong selection among the available embryos, and consequently, much effort has been put into assessing the developmental competence of individual embryos either morphologically, via genetics, kinetics, or through “omics” of various kinds [10–17]. However, in this perspective, it is important to consider the proportion of cycles in which there is no pregnancy obtained despite using all the embryos. If no biological competent oocytes are available, no selection technique or procedure will improve the outcome.

In the present study, we have calculated the cumulative live birth rate per cycle (cLBR/cycle) and the live baby born per oocyte rate (LBB/oocyte rate) for 1148 consecutive first IVF/ICSI cycles including a 5 year follow-up period to include the results from the subsequent FER cycles. In addition, it should be noted that most studies looking into live births per oocyte have looked at oocyte donation cycles, but in the present study, all data is from IVF/ICSI patients attending our clinic.

Material and methods

This study included all liveborn children resulting from a consecutive treatment cohort of 1148 fresh first cycle IVF/ICSI cycles performed in the period from January 2004 to December 2006 and including the results from the subsequent 546 frozen embryo replacement (FER) cycles with embryos resulting from this one oocyte pickup. The follow-up period of FER cycles was 5 years after the fresh transfer (e.g., December 2011), as fertilized embryos according to Danish law can be stored up to a maximum of 5 years after cryopreservation.

The women had an average age of 32.8 years ($SD \pm 3.95$ years and median 32.5 years, range 20–41.5 years) at the time of their fresh cycle. Exclusion criteria were PGD cycles or cycles where the woman or man was infected with HIV or Hepatitis. FER cycles where embryos thawed came from two different fresh cycles were also excluded from the analysis.

Ovarian stimulation was done either according to the long protocols using downregulation with gonadotropin-releasing hormone (GnRH) agonists (Synarela, Pharmacia, Denmark; Suprefact, Aventis Pharma, Denmark) for at least 14 days or according to the short protocol using GnRH antagonists

(Orgalutran, Organon, Copenhagen, Denmark; Cetrotide, MerckSerono, Copenhagen, Denmark). Patients were stimulated according to the routine practice in our clinic using recombinant follicle stimulating hormone (FSH) (Puregon, Organon; Gonal-F, Copenhagen, Serono) or urine-derived FSH (Menopur, Ferring, Copenhagen, Denmark). Ovulation was induced by urine-derived human chorionic gonadotropin (hCG) (Profasi, Serono, Pregnyl, Organon) or Ovitrelle (MerckSerono, Denmark) and oocyte aspiration was performed 36 ± 2 h after hCG administration.

The embryos were cultured in Cook sequential culture media (Cook, Sydney, Australia) and transferred 2 days after oocyte pickup. One or two embryos were transferred, and the remaining embryos of sufficient quality were cryopreserved according to the slow freezing technique. Embryos with two to six cells 48 h after oocyte pickup, less than 20 % fragmentation, and no visible multinucleated blastomeres (good quality embryos, GQE)[18] were considered of sufficient quality for cryopreservation.

FER cycles were mainly carried out in natural cycles with hCG for ovulation triggering and transfer 4 days later. In cases of anovulation or estradiol substituted cycles, hormone replacement with 6–8 mg/day of estradiol valerate and 600 mg/day of intravaginal progesterone was given and continued for 10 weeks after embryo transfer.

Intravaginal progesterone (200 mg $\times$ 3/day) was administered from the day of embryo transfer until positive pregnancy test.

Pregnancy was detected by measuring serum hCG levels 13–15 days after embryo transfer, and pregnancies with cardiac activity were confirmed by transvaginal ultrasonography at gestational week 6–7.

We had complete follow up regarding the live birth outcomes through self-reporting or for a small number via the compulsory national registers for live births. Oocyte number, number of fresh embryos transferred, embryos cryopreserved/thawed, and transferred embryos in a FER cycle were registered, and the subsequent totals were calculated. The cumulative number of live birth per cycle (cLBR), liveborn children per oocyte (LBB/oocyte rate), utilization rate (embryos transferred in the fresh cycle or frozen), and percentage of cycles with births were also calculated and compared according to women's age (women under 30, from 30–35, and above 36 years) and the level of ovarian response (1–5, 6–10, 11–15, or 16 or more oocytes).

Results

Utilization and liveborn per oocyte rates

From the 1148 cycles, we obtained 9529 oocytes, resulting in 4227 embryos of sufficient quality for either transfer or

cryopreservation (GQE), giving a utilization rate of 44.4 % (Table 1).

In total, 1416 embryos (33.4 % of the total GQE) were transferred in 1028 fresh cycle transfers (1.38 embryos per transfer) resulting in 344 liveborn children (33.5 %/cycle), and 2811 embryos (66.5 % of GQE) were cryopreserved.

In 546 subsequent FER cycles, 1867 embryos (66.4 % of those frozen) were thawed. Thawed embryos (1091) were transferred (1.81 embryos per transfer) resulting in 132 liveborn children. Consequently, 944 frozen embryos (33.6 % of the frozen embryos or 9.9 % of oocytes) were not used before the 5 years expiry date. In total, 2507 embryos were transferred in either the fresh or a FER cycle resulting in 422 deliveries and 476 liveborn children.

For each liveborn child, 5.29 embryos were transferred in either a fresh or a frozen/thawed cycle. The total number of oocytes needed per liveborn child was 20.01 meaning that only 5.0 % of the aspirated oocytes had the competence to develop into a child (Table 1). Looking only at successful cycles resulting in a live birth after either fresh or FER transfer, 12.9 % of oocytes developed into a child.

While the overall LBB/oocyte rate was 5 %, differences were found between subgroups depending on age of the women or the response to hormonal stimulation. Table 2 shows the LBB/oocyte rate according to ovarian response (1–5, 6–10, 1–15, or 16+ oocytes) and age of the woman. The number of live baby born per oocyte decreased with increasing number of oocytes retrieved for all age groups (Table 2). When

stratifying only for age, the LBB/oocyte rate dropped from 5.1 % in the <30 years age group and 5.4 in the 30–35 age group to 3.4 % in the 36+ age group (Table 2).

Cumulative live birth rates

Overall, the total cumulative live birth rate for fresh and FER cycles was 34.3 % (Table 1). In 754 of the 1148 cycles (65.7 %), no live birth was achieved despite transfer of all available embryos in either the fresh or frozen cycles. This suggests that in two out of three cycles, there were no oocytes capable of developing into a child.

Looking at the cLBR while stratifying age and number of oocytes retrieved, we find a maximal cLBR after aspiration of 11–15 oocytes, except for the age group 36+ where aspiration of 16+ oocytes resulted in the highest cLBR (Table 2). Overall, the cLBR dropped from 40.2 and 37.8 % in the two youngest age groups to 23.5 % for the 36+ group (Table 2).

Theoretical calculation on embryos leftover after 5 years of cryopreservation

In total, 944 embryos were never used. We have no information about why the couples did not return for the remaining FER treatments. We can therefore only speculate on the reasons for the patients to abandon the remaining cryopreserved embryos. Totally, 146 embryos from 42 patients were presumably abandoned due to completion of family (two or more children). Another 44 patients became pregnant in a new fresh cycle before using the frozen embryos from the study cycle. If

Table 1 Cohort overview

	Cycles resulting in a live birth (fresh or FER a)	Cycles not resulting in a live birth	Total
Cycles, <i>n</i> (% of total cycles)	394 (34.3 %)	754 (65.7 %)	1148 (100 %)
Oocytes, <i>n</i> (mean)	3694 (9.37)	5835 (7.74)	9529 (8.30)
“Good quality” embryos, <i>n</i> (% of oocytes)	1916 (51.9)	2311 (39.6)	4227 (44.4)
Embryos transferred (fresh and thawed) <i>n</i> (% of oocytes)	1004 (27.2)	1503 (25.8)	2507 (26.3)
Fresh transfers, <i>n</i>	394	634	1028
Embryos transferred, <i>n</i> (mean/transfer)	546 (1.39)	870 (1.37)	1416 (1.38)
Cryopreserved embryos, <i>n</i> (mean/transfer)	1370 (3.48)	1441 (1.91)	2811 (2.45)
LBB, <i>n</i>	344	-	344
FER cycles, <i>n</i>	276	270	546
Embryos thawed, <i>n</i> (mean/cycle)	793 (2.87)	1074 (3.98)	1867 (3.42)
Embryos transferred, <i>n</i> (mean/transfer)	458 (1.73)	633 (1.87)	1091 (1.81)
Cryopreserved embryos abandoned <i>n</i> (% frozen embryos)	577 (42.1)	367 (25.5)	944 (33.6)
LBB, <i>n</i>	132	-	132
All LBB, <i>n</i>	476	0	476
Total LBB/oocyte	12.9	-	5.0

FER frozen embryo replacement, LBB number of live babies born

Table 2 Live baby born (LBB) per oocyte rate and cumulative live birth rate according to age and number of oocytes collected

Age group Oocytes <i>n</i>	Cycles <i>n</i>	Oocytes Total, <i>n</i>	LBB <i>n</i>	LBB/oocyte %	Cycles with LB <i>n</i>	cLBR %
<30 years						
1–5	56	213	22	10.3	20	35.7
6–10	98	800	45	5.6	37	37.8
11–15	50	623	25	4.0	25	50.0
16+	20	388	13	3.4	8	40.0
All	224	2024	105	5.1	90	40.2
30–35 years						
1–5	157	582	50	8.6	44	28.0
6–10	273	2155	126	5.8	104	38.1
11 to 15	131	1648	82	5.0	65	49.6
16+	50	903	28	3.1	18	36.0
All	611	5288	286	5.4	231	37.8
36+ years						
1 to 5	115	356	16	4.5	15	13.0
6 to 10	136	1076	41	3.8	36	26.5
11 to 15	38	464	18	3.9	13	34.2
16+	18	321	10	3.1	8	44.4
All	307	2217	85	3.8	72	23.5
Total	1142 ^a	9529	476	5.0	393	34.4

LBB live babies born, LB live birth, cLBR cumulative live birth rate

^a 1142 instead of 1148 as 6 cycles had 0 oocytes retrieved

we assume that the 944 abandoned frozen embryos have the same pregnancy potential as the average frozen embryos in this study, we can calculate the remaining oocyte potential. The 1867 thawed embryos resulted in 132 children (1867/132 = 14.14 thawed embryos pr. child), consequently, 944 abandoned embryos might have resulted in additional 66 children (944/14.14 = 66.37). This would have changed the overall LBB/oocyte rate from 5.0 % to 5.7 %.

Discussion

In this study including women with an average age of 33 years, we found an overall oocyte competence rate of 5.0 %. This finding is in line with reports from other groups who have reported LBB per oocyte rates varying from 4.6 % in patients below 40 years [2] to 3.7 % in patients below 38 years (calculated from data in Table 1 published by Inge et al. [1]). Other studies have looked only at mature oocytes, finding live baby born rates of 3.8–4.5 % [5], 4.2 % [8], and 6.6 % [6] per mature oocyte. In studies looking at oocyte donors, the LBB per oocyte rate varies from 5–7.3 % [3] to 6.8 % [2] indicating only a marginal improvement in oocytes from younger women.

In oocyte cryopreservation programs, LBB per thawed oocyte rates of 4.0, 5.4, 5.9, 5.1, and 6.5 % have been reported [7, 19–22]. There are few data reporting on spontaneous cycle IVF and live birth rates suggesting an LBB of 6.60–7.2 per oocyte [23, 24].

Overall, the published data suggest that irrespective of age, maturity of the oocytes, fresh or frozen transfers in oocyte donors or IVF-patients, the LBB/oocyte rates only vary marginally between 4 and 7.3 %.

In the present study, we report a high total utilization rate of 44.4 % compared to previously reported utilization rates (31.2 % [2]; 38.4 % [25]). Utilization rates are, however, heavily dependent on the criteria for cryopreservation in individual clinics, with liberal criteria resulting in higher utilization rates than with more restrictive criteria. However, the consequence of this may only be a marginal higher LBB per oocyte rate as a more liberal freezing policy would be expected to only result in few additional pregnancies due to low implantation potential.

It should however be noted that as several embryos were sometimes frozen per straw, surplus embryos may have been discarded if there were more than two embryos surviving the thawing. This may add to a small underestimation of the total potential of the oocyte cohort.

In addition, there could be a small impact of cultural differences regarding family size between Denmark and other countries reflected in the proportion of patients not using the remaining frozen embryos after previous live births.

However, if all abandoned cryopreserved embryos had been used in this study, it would only have resulted in a marginal increase of the LBB/oocyte rate from 5.0 % to 5.7 %.

Further, in this and previous similar studies, we are unable to determine the impact of the endometrium as well as the impact of sperm quality, which both could result in an underestimation of the number of competent oocytes.

The cumulative live birth rate per cycle of 34.3 % for women aged 40 years or below as found in this study is very similar to the 33.0 % reported by Gnoth et al. [4], who also included one fresh cycle and its subsequent cryopreservation cycles.

The finding that no pregnancies were obtained in two out of three cycles may simply reflect our biology and is substantiated by the fact that women even in their most fertile period of their life do not become pregnant in every cycle despite an intention to do so. The data reported here with a pregnancy rate of 34.3 % per “cycle” is in line with the natural cycle pregnancy rate for fertile women at their most fertile period around in the early 20s [26].

This finding may call for a shift in stimulation strategies where it may be more beneficial for the patients to have several milder stimulation cycles recruiting from different cohorts of growing follicles rather than to stimulate harder in one cycle with the aim of obtaining more oocytes from the same cohort of follicles. For two out of three age groups, the cumulative live birth was highest when 11–15 oocytes were obtained in the fresh cycle. Only for the oldest population with stimulations with over 16 oocytes was the cLBR higher than with 11–15 oocytes. This may reflect the higher chance of finding a euploid embryo among a larger oocyte cohort for this older patient group [27]. It is however important to notice that the choice of stimulation regimen was based on maximizing the number of oocytes retrieved without compromising safety for the woman. Lower number of oocytes retrieved may represent an inadequate biological response to the selected stimulation strategy and dose. Our data suggests that harder stimulation (>15 oocytes) for the two youngest age groups merely increase the number of non-viable oocytes together with increased risk of ovarian hyperstimulation syndrome (OHSS) without affecting the live birth rate [1, 2, 28]. Indeed, several studies have shown that it may be preferential to go for minimal stimulation (but possibly several times) as high numbers of oocytes do not necessarily increase the number of babies per oocyte cohort [1–3, 8, 19, 29]. In the present study, the average number of oocytes retrieved was 8.30, which normally is considered in the range of mild stimulation.

In conclusion, we find that only a minor part of the retrieved oocytes after assisted reproduction have the competence to develop into a live birth of a child and in two out of

three cycles, there are no oocytes available with the necessary competence.

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