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# Total stimulation gonadotropin dose per oocyte retrieved and fresh embryo quality affect live birth rate after frozen-thawed embryo transfer

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Tiivistelmä – Referat – Abstract

Koeputkihedelmöityshoidoissa (IVF) ja munasolujen mikroinjektiohoidoissa (ICSI) elävän lapsen syntymisen todennäköisyyteen vaikuttavia tekijöitä ovat muun muassa kerättyjen munasolujen lukumäärä, saatu gonadotropiinihormonin (FSH) annos sekä luotujen korkealaatuisten alkioiden määrä. Tämän tutkimuksen tarkoituksena oli selvittää, mitkä ominaisuudet alkion tuoresiirtovaiheessa vaikuttavat syntymän todennäköisyyteen jatkossa, jos naiselle tehdään myöhemmin hedelmöityshoidossa pakastetun alkion siirto.

Tutkimusaineistona oli yhteinen Suomen lapsettomuusklinikoista kerätty tietokanta, joka sisälsi tiedot yhteensä 9465 pakastusalkionsiirto-tyyppisestä hedelmöityshoidosta vuosilta 2000-2017. Tuorealkionsiirtoja tutkittiin verraten näitä samojen naisten myöhempiin pakastealkionsiirtoihin. Luteaalisen kierron hormonaalista tukea sai naisista 42.9 %, hormonaalisesti indusoitu kuukautiskierto ja ovulaatio oli 40.7 % naisista, ja 16.1 % naisista kuukautiskierto oli täysin spontaani. FSH-annosta/munasolu käytettiin munasarjojen vasteen arviointiin.

Tutkimuksessa selvisi, että vertailussa matalin FSH/munasolu -annossuhde <200 IU/munasolu sai aikaan suurimman todennäköisyyden elävän lapsen syntymälle pakastealkionsiirron jälkeen. Korkeampi annossuhde 300-399 IU/munasolu sen sijaan laski merkittävästi tätä todennäköisyyttä. Potilaan hormonaalisesti indusoitu ovulaatiokierto niin ikään assosioitui matalampaan syntymän todennäköisyyteen kuin kierto, jossa oli annettu hormonaalista tukea vain luteaalivaiheessa. Mikäli ainakin yksi korkealaatuinen alkio oli saatavilla hoitojen alussa, oli syntymän todennäköisyys suurempi läpi koko prosessin. Naisen yli 35 vuoden ikä laski syntymän todennäköisyyttä.

Hedelmöityshoidoissa FSH/munasolu-annossuhde kuvastaa mahdollisesti munasarjojen toimintaa ja munasolujen laatua. Annossuhde tulisi pyrkiä pitämään mahdollisimman alhaisena, jotta vältytään korkeampiin annoksiin liittyvältä syntymän todennäköisyyden laskulta.

Avainsanat – Nyckelord – Keywords

frozen-thawed embryo transfer, ovarian response, embryo quality, live birth rate

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Tätä tutkimusta varten saatiin rahoitusta Sigrid Juséliuksen säätiöltä, Helsingin yliopistollisen sairaalan tutkimusrahoitusta (myönnetty J. S. Tapanaiselle) sekä Suomen Lääketieteen Säätiöltä (myönnetty Z. Velevalle). Nämä organisaatiot eivät osallistuneet tutkimuksen suunnitteluun, aineiston keräämiseen, analysointiin tai tulkintaan, eivätkä käsikirjoituksen laatimiseen tai sen julkaisupäätökseen.

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## 1 Introduction

Many factors affect live birth rate (LBR) after a frozen-thawed embryo transfer (FET). Among these, the transfer of top-quality embryos has been shown to increase LBR (1, 2). The role of endometrial preparation is also important, with hormonal substitution being associated with a lower LBR (2,3). An advanced female age at FET has been implicated in elevated miscarriage rates (4) but the effects of other characteristics on LBR after FET are not clear. For example, some studies have observed lower LBR in obese women (5) and others have shown no association (2,6).

The main factors affecting LBR after fresh IVF/ICSI are well known. These include female age, ovarian response (7), number of oocytes retrieved (8) and number of top-quality embryos created (9) but only a few analyses have focused on the effects of fresh cycle characteristics on LBR in FET. A female age ≤35 years at oocyte pickup and a mean of 15 oocytes retrieved were shown to be associated with improved LBR after FET (10) while a higher number of previous treatments has been linked with lower LBR in FET (11). High gonadotropin doses used for stimulation have been associated with poor endometrial function in fresh cycles (12) which has been the reason for the introduction of a freeze-all strategy (13,14). However, it is unclear if patients receiving high gonadotropin doses for stimulation have suboptimal outcome also in the FET cycles. In addition, a detailed analysis of LBR that evaluates simultaneously factors of the stimulation cycle and of FET is lacking.

## 2 Research aim

The aim of the present study was to find out which characteristics of the stimulation and of the FET cycles affect LBR in FET. Statistical analysis was done on data in which FET cycle data was matched with its corresponding stimulation cycle characteristics.

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# 3 Materials and methods

## 3.1 Study design

The present study is a retrospective cohort analysis of FET cycles with single embryo transfer that were performed during 2000 – 2017. Data were extracted from the LUMI database, which is a collaborative database of public and private infertility clinics in Finland that contains detailed information about autologous infertility treatments performed in Finland since 2000. For the purposes of the present study, only cycles up to the first live birth (LB) after FET per patient were collected regardless of fresh cycle outcome. Each FET cycle was matched with its corresponding fresh stimulation cycle. The following characteristics of the fresh cycle were recorded into each FET cycle data and extracted for analysis: number of oocytes retrieved, total gonadotropin dose used for ovarian stimulation, presence of tubal factor infertility, number of top-quality embryos, number of frozen embryos and LB status. In addition, we analysed the following factors of the FET cycle: age in FET, type of FET (categorised as spontaneous cycle, spontaneous with luteal support and hormonal substitution), consecutive number of FET using embryos from the same stimulation, age of the embryo at transfer (cleavage-stage, day 4, and blastocyst) as well as LB status in FET.

The main outcome variable was LB after FET. This was defined as a birth of a live infant >24 gestational weeks. The same definition was used also for the clinical outcome after a fresh transfer. Ovarian stimulation and FET cycle protocols have been previously described (4,15) and embryo culture (2,16). Briefly, ovarian stimulation was carried out using a long gonadotropin stimulating hormone agonist (GnRHa) protocol, a GnRH antagonist protocol, or a short GnRHa protocol. In spontaneous FET, embryo transfer was carried out after spontaneous ovulation was detected by means of a urinary LH test followed by ultrasound examination. In spontaneous FET with luteal support, the administration of vaginal progesterone was initiated following ovulation. Oral estradiol

was administered and the dose was adjusted until endometrial thickness was ≥7 mm, after which vaginal progesterone was initiated (hormonally substituted FET). Total gonadotropin dose per oocyte retrieved (gonadotropin dose/oocyte) was used as measure of ovarian response, and four ovarian response groups were formed (<200; 200-299; 300-399; ≥400 IU/oocyte). For all patients, tubal factor infertility status was known, and this was the only infertility diagnosis analysed, based on the results of a previous regression analysis showing that tubal factor was the only diagnosis independently affecting LBR after FET (17). The diagnosis of tubal factor infertility was made in cases with tubal occlusion at sonohysterography, laparoscopic chromopertubation, or hysteroscopy; or if the patient had a history of pelvic inflammatory disease, ectopic pregnancy and/or salpingectomy. A top-quality embryo was defined using each clinic's own scoring criteria (2,16,18). A top-quality cleavage-stage embryo was defined as having equal-sized blastomeres and no multinuclearity; four to five cells and <20% fragmentation when cultured for two days; or ≥8 cells and <20% fragmentation when cultured for three days. Blastocysts were graded according to Gardner's criteria (19).

### 3.2 Statistics

Characteristics of FET cycles with and without LB were analysed using T-test (continuous variables) or chi-square analysis (categorical variables). Gonadotropin dose/oocyte and number of top-quality embryos were analysed as both continuous and categorical variables.

Univariate logistic regression analysis on the chance of LB was performed on all variables analysed. The ones with independent effect were then entered into a general estimating equation analysis (GEE). GEE is a method of evaluating the effects of multiple variables on LB in all FET cycles of the same patient. In the analysis, the subject effect variable was the patient coded ID number, and the within-subject variable was the FET cycle coded

ID number. Variables with strong correlations and the ones without independent effect in GEE were removed from the model. Corrected Quasi likelihood under Independence Model criteria (QICC) was used to find the model with the best performance. Models with better performance have lower QICC. Analyses were performed using the IBM SPSS Statistics software, version 25. P-value <0.05 was considered to be statistically significant.

## 4 Results

In all, 9465 FET cycles with recorded pregnancy outcomes of 5905 women were analysed. Overall LBR was 20.2% (1915/9465).

Background and treatment characteristics are shown in Table 1. Women who had a LB after FET were younger than those who did not (33.1±4.2 vs. 34.2±4.2 years, P<0.0001) but had similar BMI. Patients with tubal factor infertility tended to have a lower LBR, compared to the ones with other infertility diagnoses (17.9% vs. 20.5%, P=0.06).

FET cycles with LB were observed in cases in which lower gonadotropin doses were used for stimulation than in FET cycles with no LB (1775.0±690.9 IU vs. 1847.6±741.9, P<0.0001). Furthermore, gonadotropin dose/oocyte was lower, compared to cycles with no LB (171.4±136.8 IU/oocyte vs. 186.5±145.1 IU/oocyte, P<0.0001). LBR in FET decreased from 21.5% in cycles with <200 IU/oocyte to 13.5% in the group with 300-399 IU/oocyte (P<0.0001). The number of top-quality embryos created after ovarian stimulation was higher in cycles with LB from FET (2.5±2.3 vs. 2.4±2.2, P=0.04). In cases with no top-quality embryos in the fresh cycle, LBR was 17.1% (208/1219) but if the patient had at least one top-quality embryo in the fresh cycle, LBR was significantly higher (20.7%, 1707/8246, P=0.003). The percentage of freeze-all cycles (no embryos transferred in the fresh cycle) was the same, 7.6%, regardless of LB status in FET (P=1.0).

Among characteristics of the FET cycles, spontaneous cycles with luteal support had the highest LBR of 22.7% (928/4091) and cycles with hormonal substitution – the lowest LBR (17.0%, 656/3849, P<0.0001). More FET cycles were performed to patients who did not have LB after FET than to those who did (1.5±0.9 vs. 1.4±0.9, P=0.033). The day of transfer did not affect LBR.

Table 2 shows the results of univariate logistic regression analysis. Age >35 years was associated with lower odds of LB (OR=0.62, 95% confidence interval [CI] 0.55-0.69, P<0.0001). A higher number of oocytes collected (OR=1.02, 95% CI 1.01-1.03, P<0.0001) and a higher number of top-quality embryos in the fresh cycle (OR 1.02, 95% CI 1.00-1.05, P=0.04) were associated with higher chance of LB after FET. When gonadotropin dose/oocyte <200 IU/oocyte was used as reference, the odds for LB after FET decreased to 0.79 with 200-299 IU/oocyte (95% CI 0.68-0.92, P=0.002) and to 0.57 with 300-399 IU/oocyte (95% CI 0.45-0.72, P<0.0001). Treatment year, BMI and presence of tubal infertility factor did not have independent effects on LBR.

In the final model, the number of oocytes retrieved and the number of frozen embryos were excluded from analysis because of correlation with the number of top-quality embryos. Birth in the fresh cycle was also removed due to negative correlation with dose/oocyte. In further analysis, the number of top-quality embryos was divided into two groups: patients with no top-quality embryos and patients with at least one top-quality embryo, because of lower QICC.

Results from the final GEE model are shown in Table 3. Age >35 years was associated with lower chance of LB after FET (OR 0.62, 95% CI 0.55-0.69, P<0.0001). Odds of LB also decreased in cycles with 300-399 IU/oocyte (OR 0.63, 95% CI 0.49-0.80, P<0.0001), compared to those with <200 IU/oocyte. Odds of LB after FET were higher in cases with ≥1 top-quality embryo in the fresh cycle (OR 1.29, 95% CI 1.09-1.53, P=0.003). Compared to spontaneous cycles with luteal support, hormonally substituted treatments were associated with lower chances of LB (OR 0.67, CI 0.59-0.75, P<0.0001) but the difference

was not significant for purely spontaneous cycles (OR 0.90, CI 0.77-1.04, P=0.2). A higher number of FET cycles performed was associated with lower LBR as well (OR 0.92, 95% CI 0.87-0.99, P=0.02). Transfer of blastocyst-stage or day 4 embryos had no effect on LBR compared to cleavage-stage embryo transfers.

## 5 Discussion

The present study found that LBR in FET is affected not only by the FET cycle characteristics, but also by stimulation and embryo-related factors of the fresh cycle. Among stimulation characteristics, a higher total gonadotropin dose per oocyte retrieved is associated with lower odds of LB in FET. Furthermore, the chance of LB is higher if at least one top-quality embryo is created in the fresh cycle while higher female age at FET and hormonal substitution in FET are associated with lower LBR.

A strength of the present study is the analysis of stimulation factors together with FET characteristics. This has allowed a comprehensive evaluation of the interactions between embryologic, stimulation and hormonal environment characteristics. Up to now, the effects of ovarian response on FET outcome have been scarcely studied. The observation that a higher number of oocytes retrieved was associated with a higher chance of LB in FET confirmed the findings of a small previous study of FET cycles matched with their stimulation characteristics (10). However, more data was available for analysis in the present study, including stimulation gonadotropin dose. Previously, a large retrospective analysis also demonstrated lower LBR after fresh transfers in patients having higher gonadotropin doses (20). However, in the final analysis of the present study, gonadotropin dose did not have an independent effect, most probably because similarly to the number of oocytes retrieved, it is a weaker predictive factor than stimulation dose per oocyte retrieved. Results mean that a patient who is stimulated with a total dose of 2000 IU of gonadotropins and has five oocytes has a lower chance of LB after FET compared to another patient having received the same

total gonadotropin dose but with 15 oocytes. This observation is supported by two Swedish studies investigating the effect of ovarian response on fresh cycle outcome (21,22). In these analyses, patients with high dose/oocyte (analysed as ovarian sensitivity index) were found to have lower LBR after fresh transfer.

In addition to ovarian response factors, the presence of ≥1 top-quality embryo in the fresh cycle was found to be independently associated with higher LB odds. This result is especially important for counselling patients who undergo FET after the only top-quality embryo has been transferred in the fresh cycle. Even if no top-quality embryos remain to be transferred in FET, the chance of LB is still elevated, compared to patients who had no top-quality embryos even in the fresh cycle. This observation expands the conclusions of our earlier research on the effects of top-quality embryos on the outcome of FET (2), in which presence of ≥1 top-quality embryo at each step of the freezing and thawing process until transfer in FET was associated with a stepwise increase in LBR. The present analysis also evaluated the effect on LB odds of the exact number of top-quality embryos created but found no independent association. Previous studies have evaluated the associations of the total number of embryos frozen on FET cycle outcome, which in one analysis was positively correlated with LB (10) and in another one – negatively (23). This can be explained by the different confounding factors evaluated in these analyses.

The type of endometrial preparation was the other characteristic of the hormonal environment with significant effect on LBR in FET in the present study. The analysis showed that hormonal substitution was associated with a reduced chance of LB, with OR of only 0.67, which is in line with our previous findings in a smaller data set (2). More recent studies have also demonstrated similarly suboptimal outcomes with hormonal substitution in different settings. An analysis that performed adjustment for a number of factors including embryo quality when comparing hormonal substitution to spontaneous cycles observed odds for LB that were similar to the present ones (0.71) (24). In a study of single euploid embryo transfers, LBR in patients with hormonal

substitution was almost two times lower, compared with the one in women having a spontaneous cycle (37.5 vs. 63.1%) (25). Superior LBR in ovulatory cycles may be due to the endocrine function of the corpus luteum which produces pregnancy supporting factors such as relaxin, vascular endothelial growth factor, and various angiogenic metabolites of estrogen in addition to progesterone (26,27,28). In the present analysis, results were similar in the spontaneous cycle groups regardless of luteal phase support. In Finland, during the study period, progesterone supplementation was administered whenever insufficient function of the corpus luteum was suspected. This can explain the similar results in the two spontaneous cycle groups of patients. This evidence indicates that hormonal substitution in FET should be avoided whenever possible because of the associated lower chance for LB.

Lower age in FET was another factor with independent effect on LBR in the present analysis. Advanced female age has historically been the first characteristic associated with poor success in fresh IVF/ICSI (29) and also in FET (2,30,31). However, the independent effect of age in FET in the final analysis that included also embryo quality and ovarian response factors might indicate that older age affects the chance of LB through a mechanism that is not related to oocyte or embryo quality. Previously, an oocyte donation study demonstrated lower clinical pregnancy rates with advanced recipient age (32) which might indicate poorer endometrial function in older women. However, at present there is no effective method permitting a reliable evaluation of endometrial receptivity (33) and the present observation will need to be explored in depth in future studies.

Several factors were found not to affect independently the odds for LB. Our observation that the use of freeze-all was not related to LB after FET is in contrast to previous research showing higher LBR in patients with no contraindications to fresh transfer (13, 14). In the present study, freeze-all was mainly performed to patients with increased risk of ovarian hyperstimulation syndrome (OHSS), and differences in freeze-all indications might explain the discrepancy in results. Several reports have indicated that

freeze-all might be beneficial specifically in patients with elevated progesterone (34,35). However, in Finland, during the study period progesterone levels were not routinely measured as ovarian stimulation monitoring does not rely on hormonal samples.

The present analysis found no association between day of transfer and LB in FET. This contradicts previous results showing that compared to tranfers of cleavage-stage embryos, blastocyst transfers yield better pregnancy and LB rates after frozen-thawed (36) and fresh embryo transfers (37). However, the present analysis is supported by the findings of a cohort study that observed no change in LBR after FET (38). Differences in the choice of confounding factors analysed might explain results since analyses showing better LBR with blastocyst transfer have not controlled for numbers of oocytes retrieved or gonadotropin dose. The choice of confounding factors evaluated can also explain why in the present study LB in the fresh cycle did not have independent prognostic value for LB in FET. Previously, FET cycles performed after a LB from a previous treatment have been regarded as having higher chances of LB (29,39,40) but the positive prognostic value has only been observed in studies not controlling for confounding factors. In more detailed investigations, this has not been the case as there are stronger characteristics explaining LB in FET such as embryo quality and number of oocytes retrieved (2,10).

## 6 Limitations

It was not possible to evaluate the effects of different embryo freezing methods (vitrification vs. slow freezing). As there still is no consensus on embryo quality grading after thawing, the quality of the transferred embryo was not analysed. Endometrial thickness at FET was not consistently recorded and was therefore not available for analysis, even though it is known that a thin endometrium in FET is associated with lower chances of LB (41,42,43) (Zhao et al., 2012; Bu et al., 2016, Liu et al., 2018).

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## 7 Conclusion

In conclusion, the present study showed that the outcome in FET is affected by characteristics of the fresh cycle from which the embryos originate. Total gonadotropin dose per oocyte retrieved is a variable that reflects ovarian response to gonadotropin stimulation that can be useful for anticipating poor outcome of treatment. High values of ≥300 IU/oocyte in the fresh cycle, ≥300 IU/oocyte, are associated with lower LBR in FET. In addition, a top-quality embryo in the fresh cycle is associated with higher LBR in FET, even if no more top-quality embryos remain for transfer in FET. Among the factors of the FET cycles, hormonal substitution and older female age are associated with significantly reduced odds of LB. These results are useful for patient counselling, especially in cases with poor prognosis.

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## Appendices

Table 1. Background and treatment characteristics.

| | Live birth in FET | No live birth in FET | P value |
|---|---|---|---|
| N | 1915 | 7550 | |
| Age in FET | 33.1±4.2 | 34.2±4.2 | <0.0001 |
| ≤35 years (n) | 70.3% (1273) | 59.3% (4208) | <0.0001 |
| >35 years (n) | 29.7% (538) | 40.7% (2890) | |
| BMI kg/$m^2$ | 23.8±4.1 | 23.9±4.2 | 0.3 |

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| | | | |
|---|---|---|---|
| **Factors of the fresh stimulation cycle** | | | |
| Tubal factor infertility | 9.3% (178) | 10.8% (815) | 0.06 |
| Total gonadotropin dose, IU | 1775.0±690.9 | 1847.6±741.9 | <0.0001 |
| Dose/oocyte, IU/oocyte | 171.4±136.8 | 186.5±145.1 | <0.0001* |
| <200, IU/oocyte (n) | 74.9% (1434) | 69.4% (5241) | |
| 200-299 IU/oocyte (n) | 13.0% (248) | 15.2% (1145) | |
| 300-399 IU/oocyte (n) | 4.4% (85) | 7.2% (545) | |
| ≥400 IU/oocyte (n) | 7.7% (148) | 8.2% (619) | |
| Oocytes collected | 13.6±6.1 | 13.0±6.0 | <0.0001 |
| Total number of top-quality embryos in fresh cycle | 2.5±2.3 | 2.4±2.2 | 0.04 |
| No top-quality embryos in fresh cycle | 10.9% (208) | 13.4% (1011) | 0.002 |
| 1 | 31.5% (603) | 29.7% (2239) | |
| 2 | 21.4% (409) | 20.1% (1519) | |
| 3 | 11.8% (226) | 13.9% (1050) | |
| >3 | 24.5% (469) | 22.9% (1731) | |
| Number of embryos frozen | 2.1±1.1 | 2.1±1.2 | 0.1 |
| Freeze-all | 7.6% (146) | 7.6% (575) | 1.0 |
| Birth in the fresh cycle | 19.1% (366) | 13.2% (997) | <0.0001 |
| **Factors of the FET cycle** | | | |
| Type of FET | | | <0.0001 |
| Spontaneous with luteal support | 48.5% (928) | 41.9% (3136) | |
| Hormonal substitution | 34.3% (656) | 42.3% (3193) | |
| Spontaneous without luteal support | 17.3% (331) | 15.8% (1194) | |
| FET cycle consecutive number | 1.4±0.9 | 1.5±0.9 | 0.03 |
| 1 | 41.6% (2812) | 56.4% (1026) | <0.0001 |

| | | | |
|---|---|---|---|
| 2 | 28.0% (1889) | 25.3% (460) | |
| 3 | 19.2% (1297) | 12.4% (225) | |
| >3 | 11.2% (756) | 5.9% (107) | |
| Embryo development stage at transfer | | | 0.2 |
| Cleavage-stage | 78.6% (1431) | 79.8% (5770) | |
| Blastocyst | 13.0% (236) | 11.5% (828) | |
| Day 4 | 8.7% (154) | 8.5% (629) | |

*P value from both T-test and chi-square analysis.

Table 2. Univariate logistic regression analysis for live birth after FET.

| | P value | OR | 95% CI | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Age in FET | | | | |
| >35 vs ≤35 years | <0.0001 | 0.62 | 0.55 | 0.69 |
| **Factors of stimulation cycle** | | | | |
| BMI | 0.3 | 0.99 | 0.98 | 1.01 |
| Tubal factor infertility (yes vs. no) | 0.06 | 0.85 | 0.71 | 1.00 |
| Total gonadotropin dose | <0.0001 | 0.999 | 0.999 | 0.999 |
| Dose/oocyte | <0.0001 | 0.999 | 0.999 | 1.0 |
| <200 IU/oocyte | Ref. | 1 | | |
| 200-299 IU/oocyte | 0.002 | 0.79 | 0.68 | 0.92 |
| 300-399 IU/oocyte | <0.0001 | 0.57 | 0.45 | 0.72 |
| ≥400 IU/oocyte | 0.2 | 0.87 | 0.72 | 1.06 |
| Oocytes | <0.0001 | 1.02 | 1.01 | 1.03 |
| Top-quality embryos in fresh cycle | 0.044 | 1.02 | 1.00 | 1.05 |
| No. top-quality embryos in fresh cycle | Ref. | 1 | | |
| 1 | 0.002 | 1.31 | 1.10 | 1.56 |
| 2 | 0.004 | 1.31 | 1.09 | 1.56 |

| | | | | |
|---|---|---|---|---|
| 3 | 0.7 | 1.05 | 0.85 | 1.29 |
| >3 | 0.003 | 1.32 | 1.10 | 1.58 |
| Number of frozen embryos | 0.1 | 0.94 | 0.86 | 1.02 |
| Freeze-all | 1.0 | 1.00 | 0.83 | 1.21 |
| Birth in the fresh cycle (yes vs. no) | <0.0001 | 1.55 | 1.36 | 1.77 |
| **Factors of FET cycle** | | | | |
| Type of FET | | | | |
| Spontaneous with luteal support | Ref. | 1 | | |
| Hormonal substitution | <0.0001 | 0.7 | 0.63 | 0.78 |
| Spontaneous without luteal support | 0.4 | 0.95 | 0.82 | 1.09 |
| FET cycle consecutive number | 0.03 | 0.94 | 0.88 | 0.995 |
| Embryo development stage | | | | |
| Cleavage-stage | Ref. | 1 | | |
| Blastocyst | 0.08 | 1.15 | 0.98 | 1.34 |
| Day 4 | 0.9 | 0.99 | 0.82 | 1.19 |

Table 3. Final generalized estimating equations analysis for live birth after FET.

| | P value | OR | 95% CI | |
|---|---|---|---|---|
| | | | Lower | Upper |
| Age in FET | | | | |
| >35 vs. ≤35 | <0.0001 | 0.62 | 0.55 | 0.69 |
| **Factors of stimulation cycle** | | | | |
| Dose/oocyte | | | | |

| | | | | |
|---|---|---|---|---|
| <200 IU/oocyte | Ref. | 1 | | |
| 200-299 IU/oocyte | 0.054 | 0.86 | 0.73 | 1.00 |
| 300-399 IU/oocyte | <0.0001 | 0.63 | 0.49 | 0.80 |
| ≥400 IU/oocyte | 0.4 | 0.92 | 0.76 | 1.13 |
| Top embryos in fresh cycle | | | | |
| ≥1 vs. 0 | 0.003 | 1.29 | 1.09 | 1.53 |
| **Factors of FET cycle** | | | | |
| Type of FET | | | | |
| Spontaneous with luteal support | Ref. | 1 | | |
| Hormonal substitution | <0.0001 | 0.67 | 0.59 | 0.75 |
| Spontaneous without luteal support | 0.2 | 0.90 | 0.77 | 1.04 |
| FET cycle consecutive number | 0.02 | 0.92 | 0.87 | 0.99 |