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Original Research Article

Follicle-stimulating hormone versus anti-Müllerian hormone: age related relevance to intracytoplasmic sperm injection results

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ABSTRACT

Background: Maternal age significantly impacts ovarian reserve and intracytoplasmic sperm injection (ICSI) outcomes. This study evaluated the influence of maternal age on ovarian reserve parameters and ICSI treatment results.
Methods: This prospective observational cohort study enrolled 100 infertile women (aged 20-43) undergoing ICSI in Baghdad (March 2025-February 2026), stratified by age: Group A (<35 years, n=65) and group B (≥35 years, n=35). Baseline anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), estradiol (E2), and AFC were evaluated on cycle day 2-3. All patients received a standardized fixed antagonist protocol.
Results: Women aged <35 years had significantly higher baseline AMH levels than those ≥35 years (2.06±0.94 vs. 1.51±0.82 ng/ml, p=0.004). No significant differences were found in basal FSH (p=0.876), AFC (p=0.070), or E2 (p=0.326). Women aged ≥35 required significantly higher gonadotropin doses (41.0±5.05 vs. 29.3±6.72 ampoules, p<0.001) and longer stimulation (10.4±1.09 vs. 9.63±1.32 days, p=0.003). Retrieved oocytes (6.86±3.59 vs. 7.57±3.09, p=0.302), MII oocytes (p=0.097), and fertilized oocytes (p=0.136) were lower in older women but these differences did not reach statistical significance.
Conclusions: Ovarian responsiveness declines with maternal age, requiring higher gonadotropin doses and prolonged stimulation. Serum AMH is a more sensitive marker than basal FSH for detecting early age-related ovarian decline in ICSI candidates.

Keywords: Infertility, Maternal age, Anti-Müllerian hormone, Antral follicle count, Ovarian stimulation, Assisted reproductive technology

INTRODUCTION

Infertility is defined as the inability to achieve a clinical pregnancy after 12 months of regular, unprotected sexual intercourse caused by impairment in one or both partners.[1] Over recent decades, it has evolved into a major global public health concern, affecting approximately one in six couples worldwide.[2] In assisted reproductive technology (ART), categorizing patient response as poor, normal, or high remains central to therapeutic success, yet standardized definitions remain diverse.[3] Female fecundity is intrinsically linked to chronological age because females possess a finite, non-renewing pool of oocytes established during fetal development.[4] This ovarian reserve diminishes progressively throughout life, exhibiting an accelerated depletion rate after 31.9 years of age and declining even more precipitously after age 37 due to cumulative follicular atresia and elevated rates of chromosomal aneuploidy.[5-7]

Given these biological constraints, evaluating ovarian reserve is strongly advised for women aged ≥35 years who

have experienced six months of unsuccessful attempts, as well as for younger individuals with predisposing ovarian risk factors.[8] The clinical paradigm in ICSI centers on individualizing controlled ovarian stimulation to prevent cycle cancellation or ovarian hyperstimulation syndrome (OHSS).[9] Stratification models, including the POSEIDON criteria, emphasize incorporating maternal age alongside biological ovarian markers to predict follicular recruitment accurately.[10] Although primordial follicles cannot be measured directly, indirect assessment utilizing basal FSH and AMH on cycle days 2-3, alongside transvaginal ultrasonographic antral follicle count (AFC), provides valuable clinical information.[11,15] Basal FSH levels exceeding 10-12 IU/L traditionally reflect diminished reserve, whereas AMH, synthesized by granulosa cells of preantral and small antral follicles, directly mirrors the functional follicular cohort.[13,14]

Despite the widespread clinical use of these markers, controversy persists regarding their relative sensitivity in predicting ovarian response across different maternal age strata in ICSI programs. Therefore, this prospective cohort study was conducted to evaluate the correlation between maternal age and ovarian reserve markers (specifically FSH, AMH, and AFC) and to determine their relevance to controlled ovarian stimulation dynamics and embryological outcomes in infertile women undergoing ICSI.

METHODS

Study design and setting

This was a prospective cohort study conducted from March 2025 to February 2026 at Kamal Al-Samarrai Hospital for Fertility and Sterility, Baghdad, Iraq. The study included women undergoing ICSI. Ethical approval was obtained from the hospital ethical committee prior to commencement. Written informed consent was obtained from all participants.

Study population

The study included women aged 20-43 years with primary or secondary infertility scheduled for ICSI. Inclusion criteria were: body mass index (BMI) between 20-30 kg/m², non-smokers, and no hormonal treatment in the 3 months preceding the ICSI cycle.

Exclusion criteria

Women were excluded if they had polycystic ovary syndrome (PCOS), endometriosis, pelvic pathology (including uterine fibroids or ovarian masses), congenital uterine anomalies, known autoimmune disease, diabetes mellitus, history of ovarian surgery affecting function, previous exposure to chemotherapy or radiotherapy, or recurrent implantation failure (≥3 failed ICSI cycles despite transfer of good-quality embryos).

Baseline hormonal assessment and ovarian reserve evaluation

Baseline hormonal evaluation was performed between day 2 and day 3 of the menstrual cycle in the month preceding the ICSI cycle. Serum levels of FSH, AMH, and estradiol (E2) were measured using electrochemiluminescence immunoassay (ECLIA) on Cobas analyzer (Roche Diagnostics, Germany) and expressed as IU/mL, ng/mL, and pg/mL, respectively. Antral follicle count (AFC) was assessed by transvaginal ultrasonography performed by experienced sonographers using standardized criteria.

Group stratification

Participants were stratified into two groups according to maternal age: Group A: <35 years (n=65) and group B: ≥35 years (n=35).

Controlled ovarian stimulation protocol

All women underwent controlled ovarian stimulation using a fixed antagonist protocol to minimize the risk of OHSS. Recombinant FSH (rFSH; follitropin alfa; Gonal-f, Merck Serono, Germany) was administered subcutaneously at an initial dose of 150-300 IU/day starting on day 2 of the menstrual cycle. The dose was individualized according to age, BMI, and ovarian reserve markers.

Cetrorelix acetate 0.25 mg/day (Cetrotide, Merck Serono, Germany) was administered subcutaneously starting on day 6 of stimulation. Follicular development was monitored by serial transvaginal ultrasonography, and serum E2 levels were measured when clinically indicated. The number of follicles ≥15 mm in diameter and serum E2 levels on the day of trigger were recorded.

Ovulation trigger and oocyte retrieval

Ovulation was triggered with intramuscular human chorionic gonadotropin (hCG) 10,000 IU when at least three dominant follicles reached a mean diameter of ≥17 mm. Oocyte retrieval was performed 34-36 hours after trigger under transvaginal ultrasound guidance and general anesthesia.

Women with excessively high E2 levels and/or a large number of developing follicles with concern for impending OHSS received a GnRH agonist trigger (triptorelin acetate 0.2 mg subcutaneously; Decapeptyl, Ferring, Switzerland) and were excluded from final analysis. Cycles were cancelled if fewer than three follicles ≥14 mm were present after 8-9 days of stimulation, or after an additional 4 days without meeting trigger criteria.

Fertilization, embryo transfer, and luteal phase support

Fertilization was assessed 12-24 hours after oocyte retrieval. Embryo transfer was performed on day 3 under

abdominal ultrasound guidance using a soft catheter without anesthesia. A maximum of two embryos were transferred per cycle. Embryos graded as grade I or II were considered high quality. Luteal phase support consisted of intramuscular progesterone 100 mg once daily starting on the day of oocyte retrieval, plus vaginal progesterone suppositories (Cyclogest) 400 mg twice daily.

Pregnancy assessment and follow-up

Serum β-hCG testing was performed 14 days after embryo transfer. In cases of positive pregnancy tests, transvaginal ultrasound was performed 2 weeks later to confirm clinical pregnancy, assess fetal viability, and determine the number of gestational sacs.

Outcomes

Primary outcome

Comparison of ovarian response between women aged <35 years and those aged ≥35 years, assessed by the number of oocytes retrieved.

Secondary outcomes

Comparison of ovarian reserve markers (AMH, FSH, AFC, basal E2), stimulation characteristics (total gonadotropin dose and duration), and laboratory outcomes (MII oocytes, fertilization rate, embryo transfer).

Statistical analysis

Data were analyzed using SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean±SD and compared using independent t test or Mann-Whitney U test. Categorical variables were expressed as number and percentage and compared using Chi-square test. Pearson correlation was used to assess relationships. A $p<0.05$ was considered statistically significant.

RESULTS

Baseline characteristics of the study participants

A total of 100 women were included in study. Mean age of the participants was 31.9±6.08 years, with a mean BMI of 24.6±2.52 kg/m² and a mean duration of infertility of 6.06±2.34 years. Regarding age distribution, 65 participants (65%) were <35 years, whereas 35 participants (35%) aged were ≥35 years (Table and Figure 1).

Ovarian reserve markers

The mean AMH level was 1.87±0.93 ng/ml (range: 0.01-3.43 ng/ml). The mean FSH level was 6.25±1.89 IU/ml (range: 3.02-13.6 IU/ml). The average antral follicle count was 8.04±3.87 (range: 2-16 follicles). The mean basal estradiol level was 37.2±10.1 pg/ml (range: 13.7-81.2 pg/ml) (Table 2).

Table 1: Baseline demographic and clinical characteristics of the study participants.

Variables	Mean±SD/ N (%)
Age (in years)	31.9±6.08
BMI (kg/m²)	24.6±2.52
Infertility duration (in years)	6.06±2.34
Age group (in years)	
<35	65 (65)
≥35	35 (35)

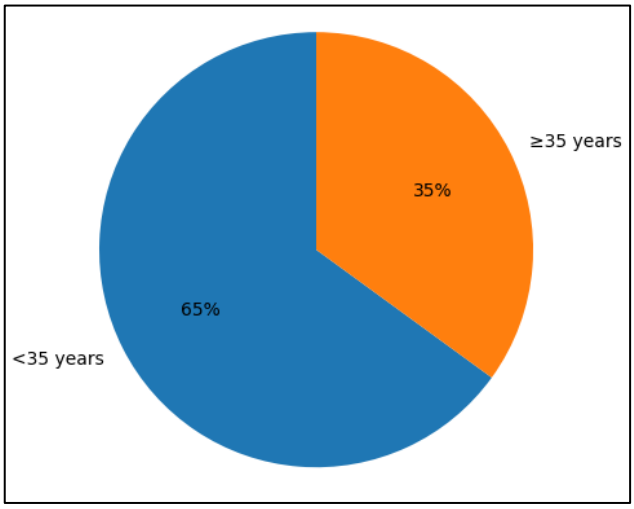

Figure 1: Age distribution of study participants.

Table 2: Ovarian reserve markers among the study participants.

Variables	Mean±SD	Min-Max
AMH (ng/ml)	1.87±0.93	0.01-3.43
FSH (IU/ml)	6.25±1.89	3.02-13.6
AFC	8.04±3.87	2-16
Basal E2 (pg/m)	37.2±10.1	13.7-81.2

Stimulation parameters and laboratory outcomes

The mean number of follicles ≥15 mm on the day of trigger was 7.80±3.51 (range: 3-16). The mean estradiol level at trigger was 787±245 pg/ml (range: 150-1205 pg/ml). Participants received a mean of 33.4±8.33 ampoules of stimulation medication, with a mean duration of stimulation of 9.91±1.30 days (range: 8-14 days).

Regarding laboratory outcomes, the mean number of oocytes retrieved was 7.32±3.27 (range: 1-14). The mean number of metaphase II (MII) oocytes was 6.00±2.91 (range: 1-12). The mean number of fertilized oocytes was 5.26±2.82 (range: 1-12). The mean number of embryos transferred was 1.92±0.31 (range: 1-3) (Table 3).

Table 3: Ovarian stimulation characteristics and laboratory outcomes of ICSI cycles.

Variables	Mean±SD	Min-Max
Follicles ≥15 mm	7.80±3.51	3-16
E2 at trigger (pg/mL)	787±245	150-1205
Ampoules used	33.4±8.33	16-52
Days of stimulation	9.91±1.30	8-14
Oocytes retrieved	7.32±3.27	1-14
MII oocytes	6.00±2.91	1-12
Fertilized oocytes	5.26±2.82	1-12
Embryos transferred	1.92±0.31	1-3

Comparison of clinical characteristics and results between the two age groups

Comparison between women <35 years and ≥35 years is shown in Table 4. BMI was not significantly different between the two groups (24.7±2.32 vs. 24.3±2.88 kg/m², p=0.446). Duration of infertility was significantly higher in the ≥35 years group (6.86±2.71 vs. 5.63±2.01 years, p=0.012).

Regarding baseline ovarian reserve, AMH levels were significantly higher in the <35 years group compared to the ≥35 years group (2.06±0.94 vs. 1.51±0.82 ng/mL, p=0.004), as illustrated in Figure 2. However, there were no significant differences between the two groups regarding baseline FSH levels (6.23±1.79 vs. 6.29±2.09 IU/mL, p=0.876), AFC (8.55±3.87 vs. 7.09±3.74, p=0.070), or basal E2 at trigger (805±224 vs. 754±280 pg/mL, p=0.326).

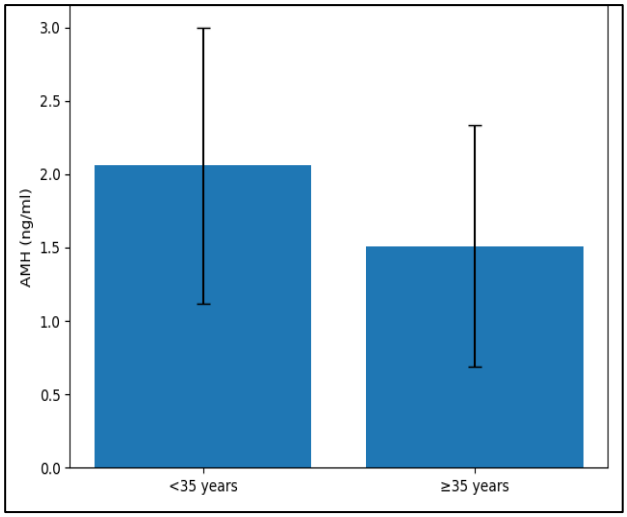

Figure 2: Comparison of AMH levels between age groups.

For stimulation parameters, the number of gonadotropin ampoules used was significantly higher in the ≥35 years group compared to the younger group (41.0±5.05 vs. 29.3±6.72, p<0.001; Figure 3). Similarly, the total duration of stimulation was significantly longer in women aged ≥35 years (10.4±1.09 vs. 9.63±1.32 days, p=0.003; Figure 4).

No significant difference was observed in the number of follicles ≥15 mm (p=0.136).

Laboratory outcomes, including retrieved oocytes (7.57±3.09 vs. 6.86±3.59, p=0.302), MII oocytes (6.35±2.92 vs. 5.34±2.80, p=0.097), and fertilized oocytes (5.57±2.84 vs. 4.69±2.72, p=0.136), were lower in the older cohort but did not differ significantly between the two groups (Table 4).

Table 4: Comparison of ovarian reserve markers, stimulation response, and laboratory outcomes between women aged <35 years and ≥35 years undergoing ICSI.

Variables	<35 years, mean±SD	≥35 years, mean±SD	P value
BMI (kg/m²)	24.7±2.32	24.3±2.88	0.446
Infertility duration (years)	5.63±2.01	6.86±2.71	0.012*
AMH (ng/ml)	2.06±0.94	1.51±0.82	0.004*
FSH (IU/ml)	6.23±1.79	6.29±2.09	0.876
AFC	8.55±3.87	7.09±3.74	0.070
Follicles ≥15 mm	8.18±3.49	7.09±3.48	0.136
E2 at trigger (pg/ml)	805±224	754±280	0.326
Ampoules used	29.3±6.72	41.0±5.05	<0.001*
Days of stimulation	9.63±1.32	10.4±1.09	0.003*
Oocytes retrieved	7.57±3.09	6.86±3.59	0.302
MII oocytes	6.35±2.92	5.34±2.80	0.097
Fertilized oocytes	5.57±2.84	4.69±2.72	0.136

*Statistically significant difference (p<0.05).

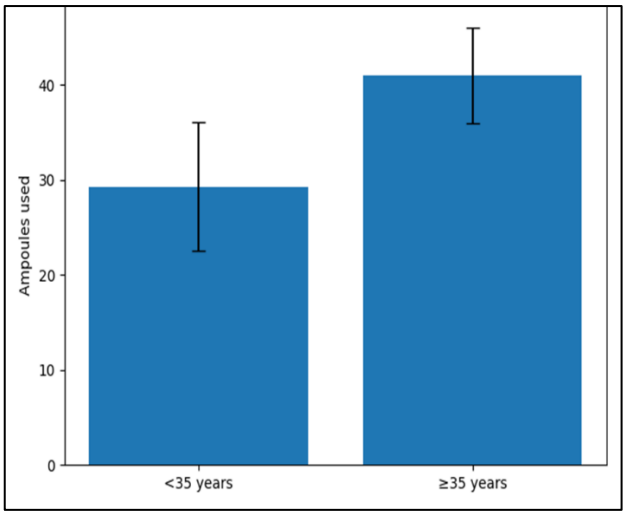

Figure 3: Stimulation medication requirements by age group.

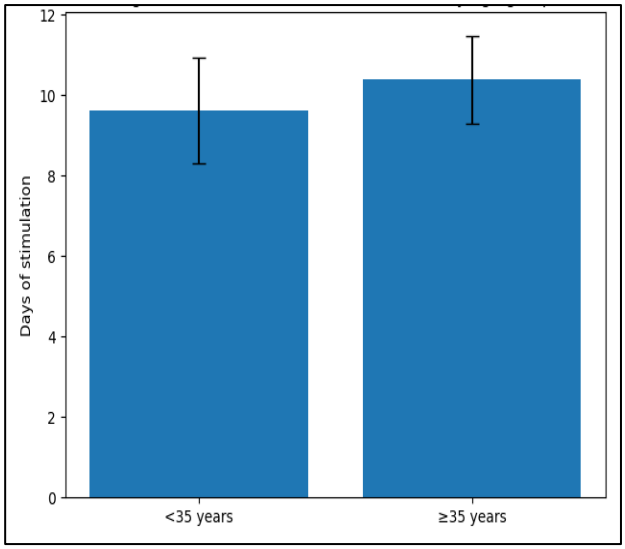

Figure 4: Duration of ovarian stimulation by age group.

DISCUSSION

The baseline demographic profile of this cohort reflects typical patterns encountered in clinical reproductive medicine worldwide. Epidemiological surveys indicate that female subfertility peaks predominantly between 30 and 39 years of age.[16] The mean BMI in our population was within the optimal physiological range, minimizing confounding metabolic impacts on ovulation and embryonic implantation.[17] Notably, the observed mean duration of infertility of 6.06 years was longer than the 1.8-3.0 years commonly reported in high-income regions.[18] This prolonged interval is characteristic of settings where healthcare-seeking behavior is delayed due to socioeconomic barriers or centralized ART facilities, which can adversely influence overall clinical prognosis.[19] Furthermore, women aged ≥35 years comprised 35% of the study group, consistent with global demographic trends toward delayed childbearing, which are often accompanied by higher frequencies of unexplained subfertility and reduced ART success rates.[20,21]

The baseline biological markers in this study indicate a relatively diminished ovarian reserve across the overall population. While the cohort mean AMH level of 1.87 ng/mL remains above the standard cutoff of 1.0 ng/ml used to identify poor ovarian response, it is substantially lower than median values recorded in healthy age-matched women, which typically range between 2.59 and 3.67 ng/ml.[22,23] The wide concentration range (0.01-3.43 ng/ml) confirms a high prevalence of reduced ovarian reserve, mirroring literature showing that over a quarter of infertile patients present with compromised reserve biomarkers.[22] Similarly, the average cohort AFC of 8.04 follicles sits below the normal benchmark of >12 follicles, with the lowest counts reaching 2 follicles, underscoring pronounced depletion in certain participants.[22,25] Baseline FSH levels remained within conventional limits (<10 IU/l) for most patients, though maximum values reached 13.6 IU/ml, which represents a recognized prognostic threshold for impaired ovarian response.[22,24]

The controlled stimulation kinetics observed in this cohort demonstrate an overall suboptimal ovarian responsiveness. The mean retrieval yield of 7.32 oocytes is considerably lower than the 15-20 oocytes established as the optimal window for maximizing cumulative live birth rates in general IVF populations.[27] This modest yield reflects the cohort's compressed baseline AMH and AFC values. In response, clinicians utilized substantial gonadotropin doses (mean 33.4 ampoules; ~337 IU/day). However, randomized evidence demonstrates that increasing daily gonadotropin dosing beyond 450 IU/day in predicted poor responders does not improve oocyte yield or ongoing pregnancy rates.[28] The mean estradiol concentration at trigger was 787 pg/ml, falling below the optimal clinical range of 1000-3148 pg/ml commonly linked with fresh cycle success.[29] In contrast, embryological performance was robust; while the oocyte maturation rate (82%) was marginally below standard benchmarks, the ICSI fertilization rate reached 87.7%, well above typical historical benchmarks of 70%-79%, indicating optimal laboratory handling and gamete preparation.[30] The clinical transfer of an average of 1.92 embryos aligns with double embryo transfer practices aimed at preserving single-cycle success rates in patients with limited oocyte yields.[31]

Comparative analysis between the age categories revealed substantial disparities in ovarian reserve and stimulation dynamics. Serum AMH concentrations were significantly depressed in women aged ≥35 years compared to younger patients (1.51 vs. 2.06 ng/ml, p=0.004), nearing the cut-off value (≤1.18 ng/ml) that predicts poor ovarian response.[32] Interestingly, basal FSH demonstrated no significant difference between the two age groups (p=0.876). This contrast underscores the clinical superiority of AMH over basal FSH for detecting early age-related ovarian reserve depletion. Because AMH is secreted continuously by preantral and early antral follicles, its circulating concentration drops proportionally with the depletion of the resting follicular pool.[32] Conversely, basal FSH elevation occurs late in the reproductive aging continuum, rising only after dramatic reductions in follicular inhibin B and estradiol compromise pituitary negative feedback.[26] Consequently, relying on normal basal FSH values in women over 35 years can provide false reassurance regarding reproductive potential.

The reduced ovarian sensitivity in women aged ≥35 years was further manifested by a 40% increase in gonadotropin consumption (41.0 vs. 29.3 ampoules, p<0.001) and a significantly longer stimulation period (10.4 vs. 9.63 days, p=0.003). These observations align with physiological data demonstrating age-related declines in granulosa cell FSH-receptor density and follicular responsiveness.[33,34] Surprisingly, however, quantitative laboratory outcomes-including oocytes retrieved, mature MII oocytes, and fertilized oocytes-showed only numerical reductions in

older women without attaining statistical significance. This finding contrasts with large-scale trials reporting an age-related loss of one oocyte every three years.[35] This discrepancy likely reflects selection bias, wherein older women accepted for ICSI had relatively preserved baseline function, combined with aggressive stimulation protocols that temporarily masked quantitative deficits.[36] Nevertheless, comparable oocyte numbers do not guarantee equivalent reproductive success; maternal age remains the overriding determinant of embryonic aneuploidy, with euploidy declining from 55% in younger women to under 30% in women over 40 years.[36] Escalating gonadotropin dosing does not reverse this intrinsic biological decline and may adversely affect embryo quality and endometrial receptivity.[37]

This study possesses several limitations that warrant consideration. The relatively modest sample size, particularly in the group aged ≥35 years (n=35), restricted statistical power to detect smaller differences in laboratory endpoints. Furthermore, conducting the study within a single specialized tertiary center in Baghdad restricts direct generalization to broader infertile populations. In addition, using a binary age threshold (<35 vs. ≥35 years) may obscure more nuanced physiological declines that could be captured using narrower age brackets. Finally, long-term reproductive endpoints, such as cumulative live birth rates and miscarriage rates, were not evaluated, limiting conclusions regarding the ultimate clinical efficacy.

CONCLUSION

This study demonstrates that ovarian reserve declines with increasing age. Women aged ≥35 years had significantly lower AMH levels and needed higher doses of gonadotropins for ovarian stimulation and longer stimulation duration compared to younger women. However, the number of oocytes retrieved, MII oocytes, and fertilized oocytes did not differ significantly between the groups.

Among the markers studied, AMH showed the strongest association with age, while FSH did not differ significantly. This suggested that AMH is a more reliable indicator of age-related changes in ovarian reserve than FSH in this cohort.

Early assessment of ovarian reserve and individualized stimulation protocols may improve outcomes in assisted reproduction, especially for women of advanced maternal age. Further studies with larger cohorts are needed to confirm these findings.

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