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Cannabis impacts female fertility as evidenced by an in vitro investigation and a case-control study

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Cannabis consumption and legalization is increasing globally, raising concerns about its impact on fertility. In humans, we previously demonstrated that tetrahydrocannabinol (THC) and its metabolites reach the ovarian follicle. An extensive body of literature describes THC's impact on sperm, however no such studies have determined its effects on the oocyte. Herein, we investigate the impact of THC on human female fertility through both a clinical and in vitro analysis. In a case-control study, we show that follicular fluid THC concentration is positively correlated with oocyte maturation and THC-positive patients exhibit significantly lower embryo euploid rates than their matched controls. In vitro, we observe a similar, but non-significant, increased oocyte maturation rate following THC exposure and altered expression of key genes implicated in extracellular matrix remodeling, inflammation, and chromosome segregation. Furthermore, THC induces oocyte chromosome segregation errors and increases abnormal spindle morphology. Finally, this study highlights potential risks associated with cannabis use for female fertility.

Cannabis consumption for both medicinal and recreational use and legalization have been rising globally[1]. Cannabis contains several classes of chemicals with cannabinoids being the most prominent; among these, tetrahydrocannabinol (THC) is the primary psychoactive compound and the most studied[2]. Notably, the concentration of THC in cannabis products has increased significantly, from an average of 3% (by weight) in the 1980s to around 15% in 2020, with some strains reaching 30% of THC[2]. The increase in frequency, ease of availability, and escalation in potency raises concerns about broader impacts on global human health, including reproductive health. Indeed, the main apprehension regarding THC and reproductive health stems from the importance of the endocannabinoid system in human reproduction[3]. Endocannabinoids, including N-arachidonoylethanolamide and 2-arachidonoylglycerol, are endogenous cannabinoids that play a central role in both male and female reproduction[3], whereas THC is an exogenous cannabinoid. Extensive research has documented the effects of THC on male reproduction, highlighting an impact on sperm deoxyribonucleic acid (DNA) methylation[4–7] and sperm parameters[8] including sperm concentration[9–11], morphology[12–14] and motility[14]. As for female health, literature reports the impact of cannabis use during pregnancy on pregnancy outcomes[15–18], placental development[18–20] and offspring health[18,20–22]. However, to our knowledge, no studies have investigated the impact of cannabis on the human female gamete, the oocyte, a gap partly due to the challenge associated with obtaining these samples.

During in vitro fertilization (IVF) treatment, exogenous gonadotropins are administered in a process called "controlled ovarian hyperstimulation" which recruits multiple follicles and induces follicle growth. These recruited follicles, each containing an oocyte, are

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then collected by a physician in a procedure called oocyte retrieval. Oocytes are collected along with their surrounding microenvironment, including follicular fluid (FF) and supportive somatic cells (granulosa cells). The oocytes are isolated, and mature oocytes are used for subsequent in vitro fertilization. Using FF, our group has previously quantified Δ9-THC and its metabolites, 11-OH-THC and 11-COOH-THC[23,24], demonstrating that these compounds could reach the follicular niche. This is significant as it suggests that THC may directly alter the microenvironment where the oocyte matures. Furthermore, our group has shown that THC exposure altered human granulosa cell methylation in a concentration dependent manner[23], and in vitro exposure modulated cannabinoid receptor dynamics in granulosa cells[24]. However, no human studies and only a few animal model studies have investigated the impact of cannabis directly on oocyte development with conflicting results[25–29].

Maturation of the oocyte is a unique and highly specialized process beginning in utero during fetal development. It is widely accepted that female neonates are born with a finite number of oocytes, which, following menarche, are recruited to mature in cohorts with each menstrual cycle[30]. Although oocytes are protected in the ovary by the blood-follicle-barrier, they remain highly sensitive to environmental factors[31]. Given their essential role in reproduction, any perturbations in their development and maturation could have profound effects on fertility and on future generations. Thus, understanding the impact of THC on oocyte health is critical for providing informed guidance and counseling to patients of the potential risks to their fertility and future offspring.

In this study, we determine the impact of physiologically relevant concentrations of THC on oocyte maturation, elucidate the transcriptomic changes induced by THC exposure and its effect on chromosome segregation, and compare our findings with a retrospective cohort study. Our investigation will aid in bridging the knowledge gap in our understanding of the sex-specific reproductive consequences of cannabis use and contribute to more effective and evidence-based patient counseling.

Results

THC concentrations correlate with maturation rate in IVF

Using a retrospective case-control design and mass spectrometry, we quantified the concentration of Δ9-THC and its metabolites,11-OH-THC and 11-COOH-THC, in the FF of patients undergoing IVF treatment to determine the reproductive consequences of THC consumption. Figure 1a illustrates the proportion of THC and its metabolites measured in all samples ($n = 1059$). Positivity rate was defined by the presence of 11-COOH-THC in the follicular fluid (62/1059, 6%). 11-COOH-THC was found alone in 13% of the samples (8/62) while Δ9-THC was co-detected in 37% of the samples (23/62) and 11-OH-THC co-detected in 2% (1/62). All three compounds were measured in 48% of the samples (30/62). Among the positive patients, 73% did not disclose their THC consumption on the patient intake questionnaire. The distribution of Δ9-THC and its metabolites showed a predominance of 11-COOH-THC (mean = 28.8 ng/mL), followed by Δ9-THC (mean = 7.5 ng/mL), with 11-OH-THC being the least abundant (mean = 1.7 ng/mL) (Fig. 1b). Notably, concentrations of these metabolites did not differ between FF and matched serum samples obtained at the time of oocyte retrieval (Fig. 1c).

A Spearman correlation analysis identified significant correlations between THC metabolite concentrations and various clinical and biochemical parameters (Fig. 1d). Specifically, concentrations of Δ9-THC, 11-OH-THC and 11-COOH-THC were positively correlated with oocyte maturation rate in the THC-positive group (Δ9-THC: $\rho = 0.370$, $p = 0.003$; 11-OH-THC: $\rho = 0.309$, $p = 0.014$ and 11-COOH-THC: $\rho = 0.295$, $p = 0.020$). Interestingly, Δ9-THC levels were negatively correlated with a patient's Body Mass Index (BMI) ($\rho = -0.539$, $p = 0.000053$).

In vitro THC exposure and oocyte maturation

Patients undergoing IVF treatment and oocyte retrieval who consented for the collection of IVF waste material (immature oocytes, somatic cells and FF) and de-identified clinical data were included in this study. For each patient, a minimum of three immature oocytes at the germinal vesicle (GV) stage were collected following the removal of somatic cells. GV oocytes were cultured using our standard in vitro maturation (IVM) protocol for 24h[32] (control group (Ctrl), $n = 96$) or with the addition of THC (treatment groups). Oocytes were treated with either a physiologically relevant (THC1, $n = 95$, 25 ng/mL Δ9-THC, 5 ng/mL 11-OH-THC, 50 ng/mL 11-COOH-THC) or a supraphysiologic (THC2, $n = 93$, 100 ng/mL Δ9-THC, 50 ng/mL 11-OH-THC, 200 ng/mL 11-COOH-THC) concentration where THC1 is based on the concentration of Δ9-THC and its metabolites measured in the follicular fluid of IVF patients and THC2 is based on previously reported concentrations in animal studies[23,25,29]. Subsequently, oocytes were classified based on their progression through key maturation checkpoints: germinal vesicle (GV) and Metaphase-I (MI) (after germinal vesicle breakdown (GVBD) and before polar body extrusion) were considered immature oocytes, while Metaphase-II (MII) oocytes (after visible polar body extrusion) were considered mature (Fig. 2a). Maturation rate was then calculated per treatment group.

Oocytes treated with THC1 showed no significant change in maturation rate (49/95, 52%, $p = 0.6704$), while THC2 exhibited a non-significant trend toward increased maturation (54/93, 58%, $p = 0.1098$), compared to Ctrl (44/96, 46%) (Fig. 2b). Utilizing timelapse imaging, oocyte morphology assessments were performed pre-IVM (Ctrl: $n = 92$, THC1: $n = 89$ and THC2: $n = 85$) and post-IVM (Ctrl: $n = 91$, THC1: $n = 88$, and THC2: $n = 83$), and key maturation events were recorded: GVBD (Ctrl: $n = 71$, THC1: $n = 72$ and THC2: $n = 64$) and extrusion of the first polar body (Ctrl: $n = 28$, THC1: $n = 30$ and THC2: $n = 31$). Examples of timelapse IVM images are provided in Supplementary Fig. 1 and corresponding videos are provided as Supplementary videos (Ctrl-Supplementary video 1, THC1-Supplementary video 2 and THC2-Supplementary video 3). There were no significant differences in oocyte diameter between treatment groups either before (Ctrl: 110.6 µm, THC1: 109.6 µm, $p = 0.2120$ and THC2: 109.6 µm, $p = 0.2120$) (Fig. 2c) or after 24 h of culture (Ctrl: 110.2 µm, THC1: 110.0 µm, $p = 0.7416$ and THC2: 108.8 µm, $p = 0.1066$). (Fig. 2d). Similarly, the timing of GVBD (Fig. 2e) and polar body extrusion (Fig. 2f) remained unaffected by THC exposure. Demographic data of patients included in these analyses can be found in Supplementary Information - Supplementary Table 1.

THC exposure alters the oocyte transcriptome

Single MII oocytes with good morphology and normal developmental progression were sequenced using our optimized ultra-low input RNA sequencing pipeline[33] ($n = 24$ patients/$n = 86$ metaphase-II (MII) oocytes (28 Ctrl, 27 THC1 and 31 THC2). Differential expression analysis revealed 89 genes up-regulated and 227 genes down-regulated greater than 2-fold ($|\log_2 FC| > 1$) and $p < 0.05$ (Fig. 3a) when assessing the impact of the THC1 vs Ctrl (Supplementary Data 1). Gene Set Enrichment Analysis (GSEA) identified that upregulated genes were principally associated with positive regulation of synaptic transmission, axonemal dynein complex assembly, and glutamate receptor signaling pathway, while the downregulated genes were associated with protein synthesis, expression regulation of SLITS and ROBOS and inflammatory processes (Fig. 3b, Supplementary Data 3). THC2 exposure induced a greater magnitude of transcriptomic dysregulation, with 402 up-regulated and 62 down-regulated genes identified (Fig. 3c, Supplementary Data 2). The upregulated genes were associated with the immune system and apoptotic pathways while downregulated genes were associated with attachment of spindle microtubules to kinetochores and inflammatory processes (Fig. 3d, Supplementary Data 3). As illustrated by the

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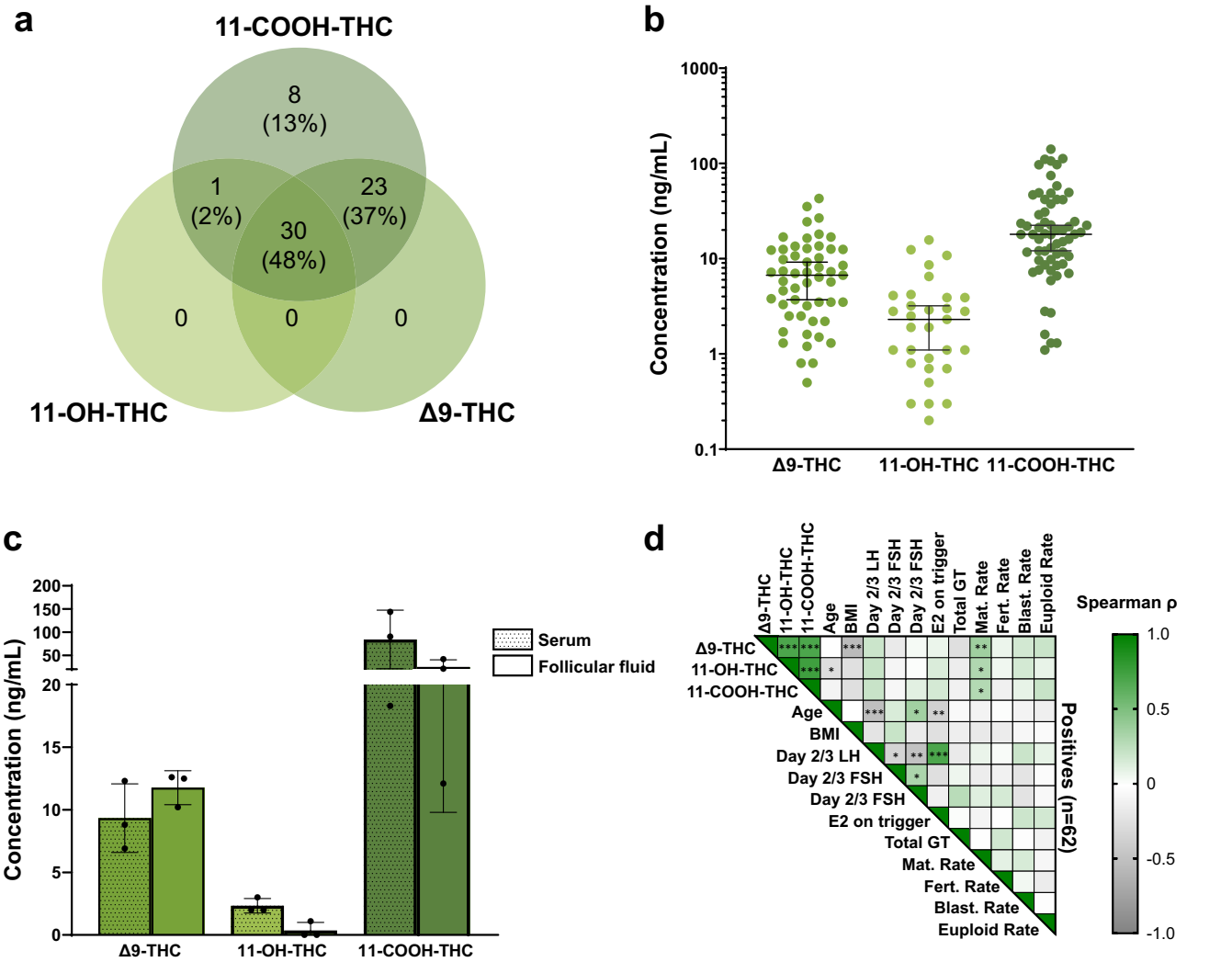

Fig. 1 | Tetrahydrocannabinol concentrations and correlation with demographic data and clinical in vitro fertilization outcomes. a Proportion of follicular fluid (FF) samples positive for Δ9-THC, 11-OH-THC and 11-COOH-THC (presence of 11-COOH-THC=positive sample). **b** Distribution of Δ9-THC ($n = 53$), 11-OH-THC ($n = 31$), and 11-COOH-THC ($n = 62$) concentrations in FF (data presented as median with interquartile range) and **c** concentrations of Δ9-THC, 11-OH-THC, and 11-COOH-THC in FF and matched serum samples (data presented as mean ± standard deviation, from $n = 3$ individual participants with matched serum samples (dotted fill pattern) and FF samples (plain)). **d** Correlation matrix of clinical and biochemical parameters in THC-Positive group ($n = 62$). The colors represent the two-sided Spearman ρ value $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. AMH Anti-Müllerian Hormone, BMI Body Mass Index, LH Luteinizing Hormone, FSH Follicle Stimulating Hormone, E2 Estradiol, GT Gonadotropins, THC Tetrahydrocannabinol, Mat. Rate Oocyte maturation rate, Fert. Rate Fertilization rate, Blast. Rate Blastulation rate. Source data are provided as a Source Data file.

Venn diagram (Fig. 3e), THC1 exposure had 266 specific DEGs, while THC2 exposure had 414, with 50 being common to both treatment groups (gene lists are available in Supplementary Data 4). Of these 50 common DEGs, 32 were protein-coding, 19 were up-regulated (*EPYC, RGS5, N4BP2L1, KRT19, PRR20G, KL, KCND3, ALDH3A1, SLC1A3, TSPAN8, PLAU, COL8A2, TFAP2E, SPTSSB, BRINP3, VANGL2, RGS18, RXFP1*, and *KCNMB3*), 10 were down-regulated (*OR4F15, MMP9, PRRX2, IRS4, INFG, CCIN, IL33, NEUROD1, MT1HL1*, and *MT1H*), and 3 displayed bidirectional changes (*S100B, ACTA1*, and *ARHGEF19*) (Fig. 3f) (Detailed information available in Supplementary Data 5). Demographic data of patients included in these analyses can be found in Supplementary Information - Supplementary Table 2 and Sequencing Quality Control metrics can be found in Supplementary Data 6.

THC is harmful to chromosome segregation

Subsets of MII oocytes from both the control and THC-treatment groups were used to assess polar body ploidy status (18 Ctrl, 21 THC1, and 21 THC2) and for spindle morphology (12 Ctrl, 12 THC1, and 12 THC2). Removal of the zona pellucida (ZP) and subsequent polar body biopsy (Supplementary Fig. 2) allowed for some oocytes to be used for ploidy determination by low-pass whole genome Next-Generation Sequencing (NGS) aneuploidy using VeriSeq PGT-A which is specialized in detecting aneuploidy in reproductive samples[21,34] (Supplementary Fig. 3) and meiotic spindle organization by confocal microscopy allowing for precise visualization of spindle organization and chromosome alignment (Fig. 4a). Both THC1 and THC2 treatment led to a 9% increase in aneuploidy (Ctrl: 39%, THC1 and THC2: 48%, $p = 0.7479$) (Fig. 4b) and a higher proportion of complex aneuploidy, defined by the gain or loss of more than three chromosomes[35] (Ctrl: 0%, THC1 and THC2: 42%, $p = 0.1029$) (Fig. 4c). Figure 4d reports a subset of oocytes where both ploidy status and spindle morphology were assessed ($n = 17$), without stratifying by treatment group. The majority of oocytes that completed meiosis I displayed normal spindle morphology (euploid: $n = 8/13$, 62% and aneuploid: $n = 3/4$, 75%, $p > 0.9999$) (Fig. 4d), but not all. The hallmark characteristics of

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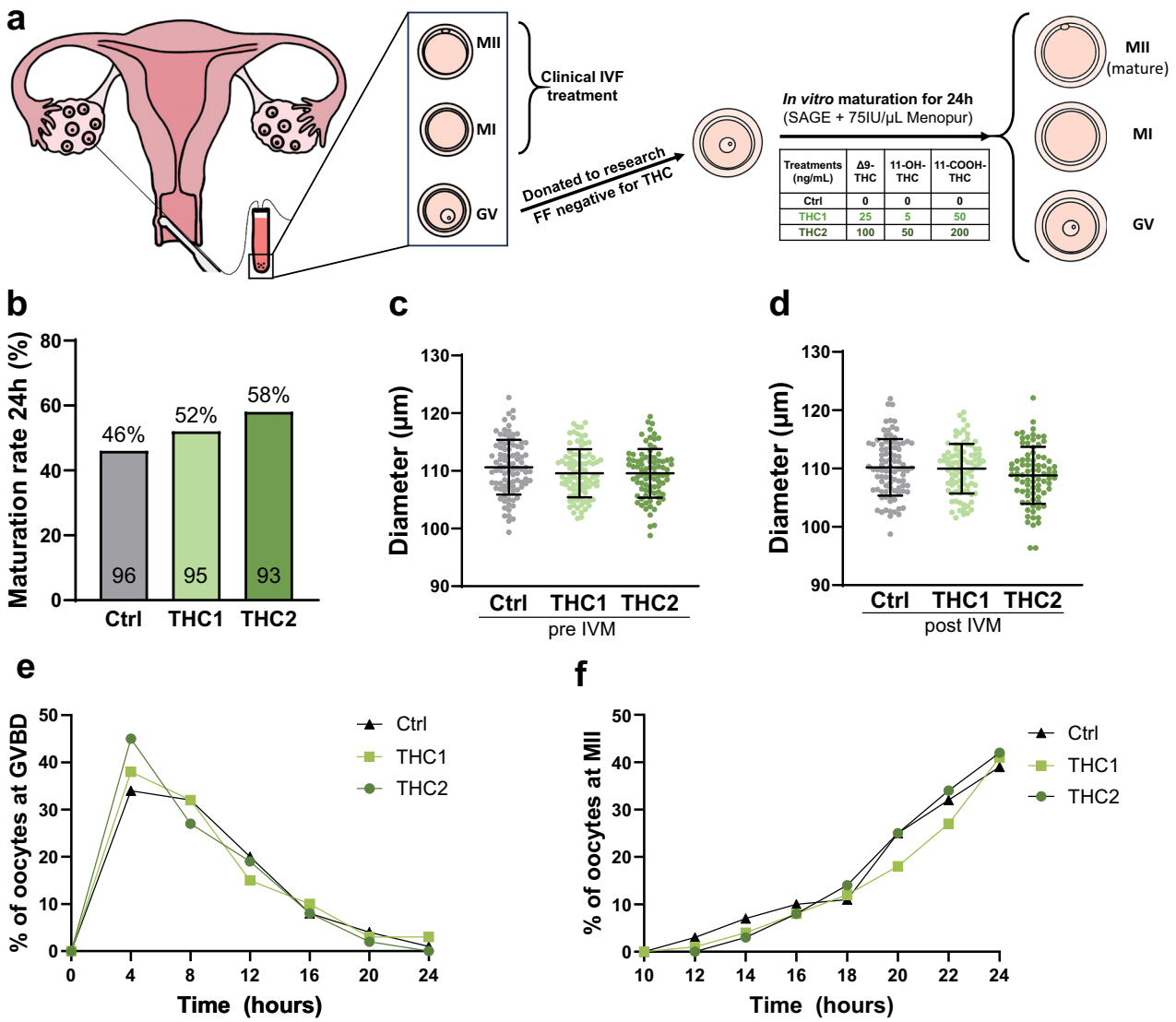

Fig. 2 | Impact of tetrahydrocannabinol exposure on oocyte maturation. **a** Experimental design and in vitro oocyte maturation. **b** Maturation rate as the percentage of germinal vesicle (GV) oocytes that matured (progressed to metaphase-II (MII)) rate (Ctrl (44/96, THC1: 49/95, THC2: 54/93). Oocyte diameter (**c**) prior to exposure to THC (Ctrl: $n = 92$, THC1: $n = 89$, THC2: $n = 85$) and **d** after 24 h of culture (Ctrl: $n = 91$, THC1: $n = 88$, THC2: $n = 83$). Proportion of oocytes that underwent key maturation events after 24 h of culture: **e** germinal vesicle breakdown (GVBD) (Ctrl: $n = 71$, THC1: $n = 72$, THC2: $n = 64$) and **f** extrusion of the first polar body (MII-arrested stage) (Ctrl: $n = 28$, THC1: $n = 30$, THC2: $n = 31$). Error bars represent the mean ± standard deviation. Significance was assessed by two-sided Fisher's exact test or One-way ANOVA with two-sided Holm–Sidak multiple comparison test (ns not significant). IVF In vitro fertilization, IVM In vitro maturation, MII metaphase II, MI metaphase I, GV germinal vesicle, GVBD germinal vesicle breakdown, FF follicular fluid, THC tetrahydrocannabinol. Source data are provided as a Source Data file.

"normal" meiotic spindles include bipolar barrel-shaped microtubules with the chromosomes aligned on the metaphase plate[36]. Whereas "abnormal" spindles are varied in their morphology and may include multipolar spindles, alterations in microtubule organization, and misaligned chromosomes[36]. Spindle disorganization and chromosome misalignment are shown by representative images in Fig. 4e, where oocytes were classified as having either "normal" or "abnormal" spindles. The proportion of oocytes with abnormal spindles was higher in the THC exposed groups compared to control (Ctrl (5/12), THC1 8/12), and THC2 (11/12), with a significant increase in THC2 (Ctrl: 42% and THC2: 92%, $p = 0.0272$) (Fig. 4f). (Spindle immunostaining negative controls ca be found in Supplementary Fig. 4)

THC decreases embryo euploidy rate in IVF

Following pairwise case-control matching, where each THC-positive sample was matched to two THC-negative samples, a significant decrease in embryo euploidy rate was observed in the THC-positive group ($n = 51$, 60.0%), compared to the THC-negative group ($n = 101$, 67.0%, $p = 0.0245$) (Table 1). There was no significant change in maturation, fertilization and blastocyst rates (Table 1).

To further evaluate the likelihood of adverse IVF outcomes, we conducted multiple logistic regression analyses, focusing on clinically relevant IVF outcome thresholds[37]: maturation rate (80%), fertilization rate (70%), blastocyst rate (50%) and euploidy rate (60%). We utilized backward stepwise logistic regression, including the following covariates: oocyte age, participant body mass index (BMI), anti-müllerian hormone (AMH), day 2/3 luteinizing hormone (LH), and follicle stimulating hormone (FSH), (estradiol) E2 on trigger, and total gonadotropin (GT) dose. The final model for both blastulation and euploidy rates retained THC status as a significant explanatory variable, with oocyte age being a significant covariate. In this pairwise matched cohort, THC positivity significantly

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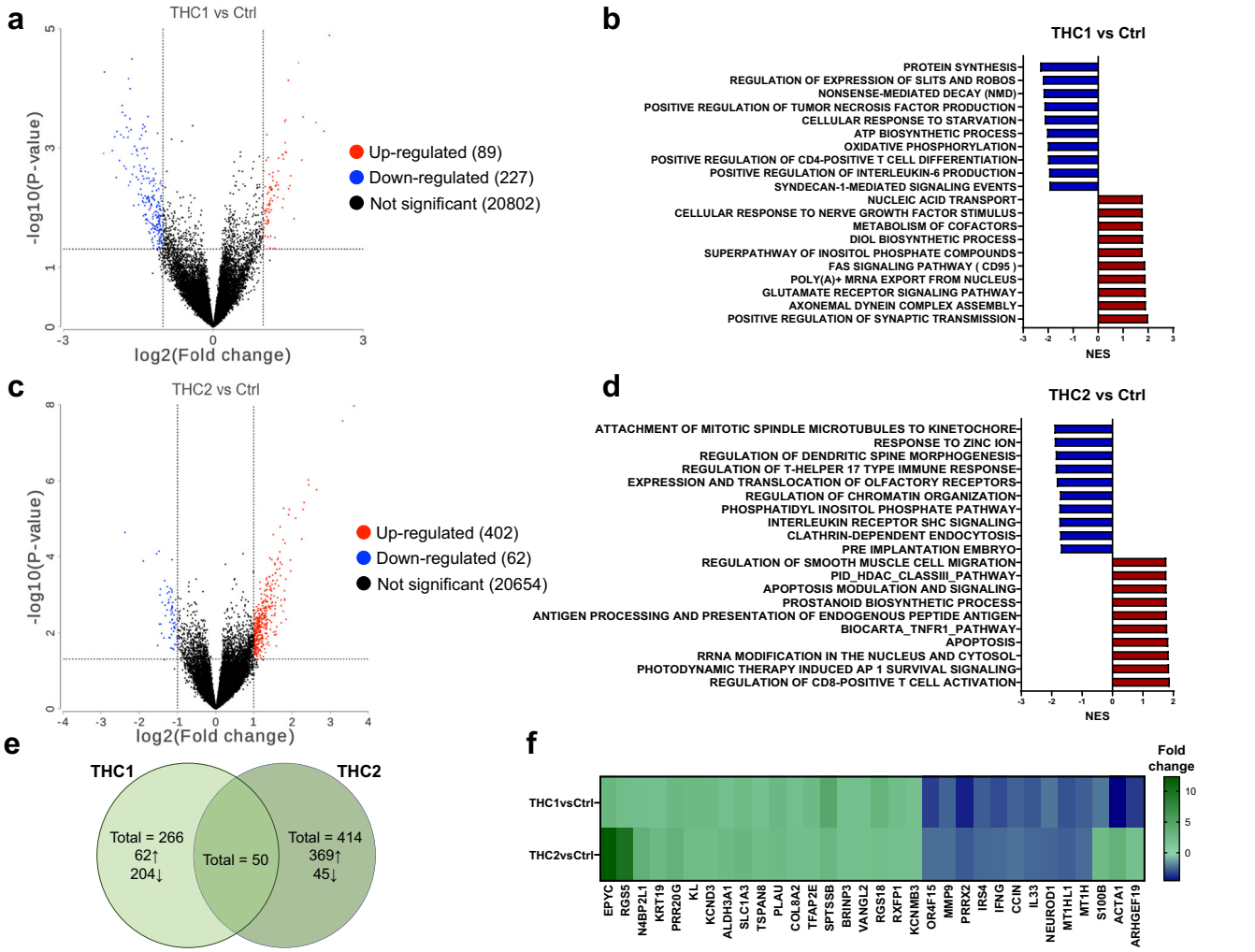

Fig. 3 | Effect of tetrahydrocannabinol exposure on oocyte transcripts after oocyte in vitro maturation. Volcano plots of differentially expressed genes (DEGs) comparing (**a**) THC1 vs Ctrl and **b** pathway analysis by gene set enrichment analysis (GSEA) THC1 vs Ctrl. Volcano plots of DEGs comparing **c** THC2 vs Ctrl and **d** GSEA of the comparison THC2 vs Ctrl. **e** Venn diagram of DEGs in THC1 and THC2 compared to Ctrl. **f** Common protein-coding DEGs, colors representing fold change. DEGs were defined by $p < 0.05$ (twp-sided Wald test resulting in unadjusted p-values) and $\log_2$fold change ($|\log_2 FC| > 1$, $n = 24$ patients/$n = 86$ metaphase-II (MII) oocytes (28 Ctrl, 27 THC1 and 31 THC2). Significant pathways were defined as having a normalized enrichment score ($|NES| > 1.5$ and $p < 0.05$ (two-sided permutation test resulting in unadjusted p-values).

decreased the odds of reaching a 50% blastulation rate or above (odds ratio: 0.45, $p = 0.018$) and the odds of achieving a euploidy rate above 60% (odds ratio: 0.47, $p = 0.038$) (Table 2). Age was also found to significantly impact blastulation and euploidy rates, with an odds ratio of 0.9144 ($p = 0.0010$) and 0.9150 ($p = 0.0024$), respectively. The models for predicting blastulation rate (>50%) and euploidy rate (>60%) demonstrated positive predictive power, with areas under the curve (AUCs) of 0.68 and 0.67, respectively (Supplementary Fig. 5).

Discussion

Understanding the impact of environmental factors and lifestyle choices on female fertility is crucial for proper patient counseling. With cannabis being one of the most commonly used recreational drugs in the world[1], it is critical to holistically evaluate its impact on mental and general health, in addition to reproductive health. This study, using donated human oocytes and an integrated multidisciplinary approach, reveals that exposure to THC affects oocyte maturation, transcriptome, and induces meiotic chromosomal imbalances associated with altered spindle morphology. Moreover, our retrospective analysis revealed that exposure to THC was associated with significantly lower embryo euploidy rate, likely partially explained by an altered chromosomal organization as demonstrated in the in vitro matured MII oocytes.

In our retrospective study, we measured THC concentrations in 1059 follicular fluid samples from patients undergoing IVF treatment at CReATe Fertility Centre (Toronto, Canada) in a retrospective matched case-control cohort. Sixty-two samples tested positive for 11-COOH-THC, resulting in a 6% positivity rate (Fig. 1a). This rate is considerably lower than what was reported by a recent Health Canada survey where 23% of females reported recreational cannabis consumption within 1 year of being surveyed[38]. However, these patients were counseled pre-treatment not to use recreational drugs while undergoing IVF. The relative concentrations of Δ9-THC, 11-OH-THC, and 11-COOH-THC are consistent with metabolism of THC in the liver (Fig. 1b). Δ9-THC (half-life 1.3–10 days) is metabolized to 11-OH-THC (half-life 20 min–2 h) and 11-OH-THC is rapidly metabolized to 11-COOH-THC (half-life 3–5 days), which remains in the circulation for up to 30 days[39]. The consistent concentrations of THC metabolites in both the follicular fluid and serum suggests passive diffusion or transudation from the bloodstream into follicular fluid rather than active transport into or out of the follicle (Fig. 1c).

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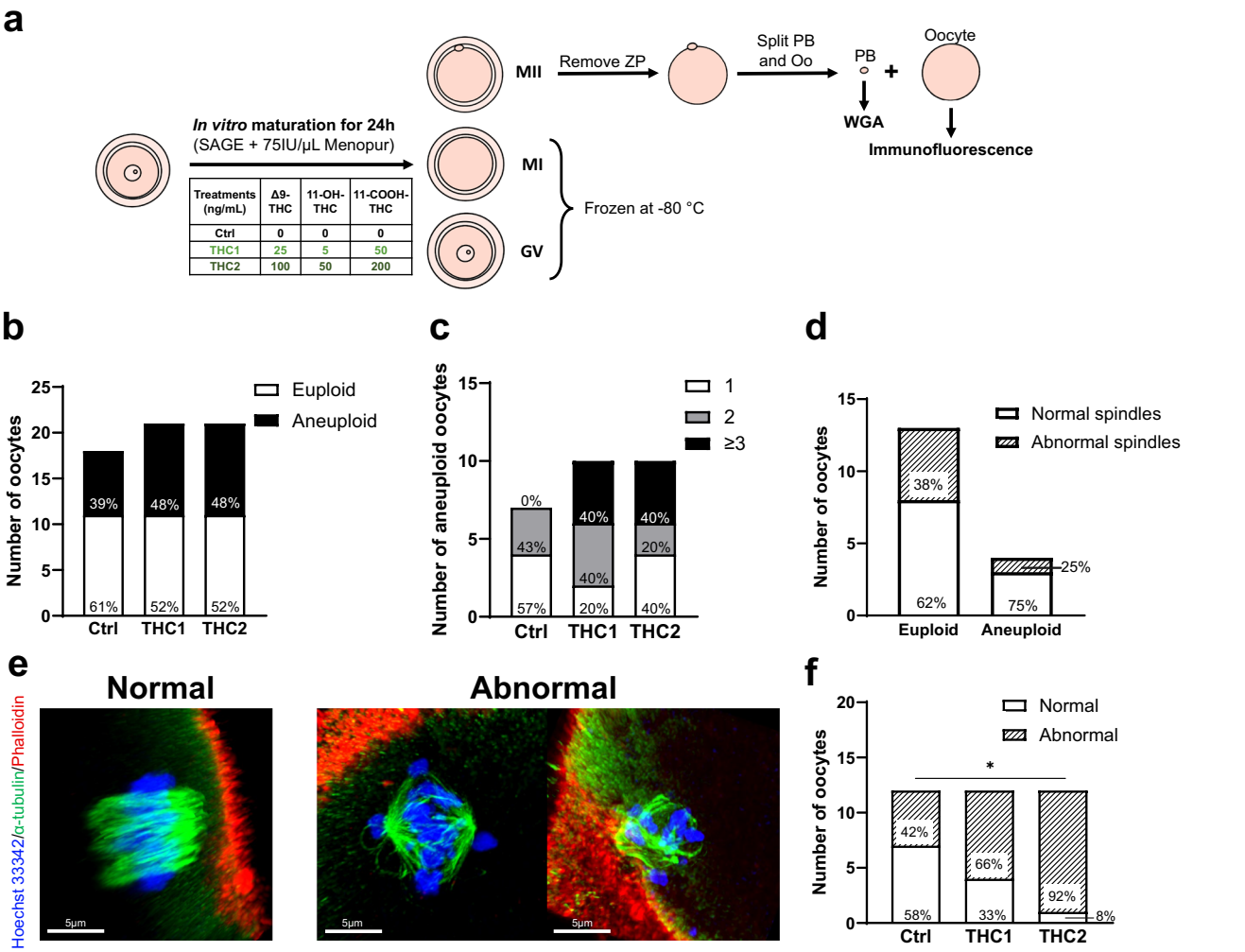

Fig. 4 | Impact of tetrahydrocannabinol exposure on oocyte spindle morphology and ploidy status. a Experimental design for polar body sequencing and oocyte immunostaining. **b** Proportion of euploid oocytes deduced by polar body biopsy sequencing results in Ctrl (11/18), THC1 (11/21), and THC2 (11/21). **c** Proportion of aneuploid oocytes with a gain/loss of one chromosome (white), two chromosomes (gray) or three and more (black) in Ctrl (1 chromosome: $n = 4$, 2 chromosomes: $n = 3$, ≥3 chromosomes: $n = 0$, total $n = 7$), THC1 (1 chromosome: $n = 2$, 2 chromosomes: $n = 4$, ≥3 chromosomes: $n = 4$, total $n = 10$) and THC2 (1 chromosome: $n = 4$, 2 chromosomes: $n = 2$, ≥3 chromosomes: $n = 4$, total $n = 10$). **d** Proportion of normal morphology spindles in euploid (8/13) and aneuploid (3/4) oocytes. **e** Representative images of normal ($n = 12$) and abnormal ($n = 24$) metaphase-II-arrested oocyte spindles, chromosomes (Hoechst)are in blue, spindles (α-tubulin) are in green and cell membrane (Phalloidin) in red. Scale bar: 5 µm. **f** Proportion of oocytes with abnormal spindles in Ctrl (5/12), THC1 (8/12), and THC2 (11/12, $p = 0.0272$). Significance was assessed using two-sided Fisher's exact test. GV germinal vesicle, MI metaphase I, MII metaphase II, Oo oocyte, PB polar body, THC tetrahydrocannabinol, WGA whole genome amplification, ZP zona pellucida. Source data are provided as a Source Data file.

In a clinical IVF setting, embryologists and physicians assess mature oocytes based on the extrusion of the first polar body[30,40]. This process, known as nuclear maturation, marks the oocyte's progression to metaphase-II and is considered the final stage of oocyte maturation[30,41]. When we compiled the IVF outcomes of our retrospective cohort, we observed a positive correlation between THC metabolites and oocyte maturation (Fig. 1d). This suggests that the levels of THC and its metabolites present in this cohort may support nuclear maturation through an unknown mechanism.

Parallel to our clinical retrospective study, we treated pairwise-matched immature oocytes from patients who were negative for THC (naive GVs) with THC in vitro and we observed an increased proportion of oocytes which achieved the MII stage (Fig. 2b). Given that factors such as oocyte diameter have been previously reported to influence nuclear maturation[42], we confirmed that there were no differences in oocyte size distribution across the three groups (Ctrl, THC1 and THC2) before and after culture for 24 h (Fig. 2c, d). Finally, we also monitored and recorded the timing of key maturation events using timelapse imaging technology and showed that the GVBD occurred slightly faster in THC treated oocytes (Fig. 2e), but this did not reach significance, and there were no differences in the timing of the first polar body extrusion between THC-exposed and Ctrl groups (Fig. 2f). Previous animal studies investigated the impact of THC on oocyte maturation using bovine oocytes[29], which is considered a better proxy to human oocyte maturation when compared to murine model due to similar temporal dynamics. López-Cardona et al. reported a 15% increase in maturation rate after treating oocytes with 0.1 µM (31.45 ng/mL) Δ9-THC for 12 h ($n = 30$/group)[29]. In contrast, a more recent and larger study ($n = 164$/group) concluded that a 24 h treatment with 'recreational cannabis doses' of 0.32 µM (100.63 ng/mL) and 3.2 µM (1,006.30 ng/mL) of Δ9-THC significantly reduced oocyte maturation from 80.1% to 65.3% and 60.1%, respectively[25]. Of note, the latter study used higher doses of Δ9-THC than what is measured in the FF of our patients but neither study examined the combined effects of Δ9-THC and its metabolites, which might alter its effect on the growing oocyte. Collectively, our results suggest that THC exposure, at both

Table 1 | Demographic data and in vitro fertilization outcomes of pairwise matched patients negative and positive for tetrahydrocannabinol

		Negative (*n* = 124) Median (IQR)	Positive (*n* = 62) Median (IQR)	*p*-value
THC concentrations	Δ9-THC (ng/mL)	–	5.3 (9.6)	
	11-OH-THC (ng/mL)	–	0.1 (2.3)	
	11-COOH-THC (ng/mL)	–	18.1 (29.1)	
Age (years)[a]		30.0 (9.0)	29.5 (11)	0.4863
BMI (kg/m^2)		23.4 (4.8)	24.3 (8.3)	0.9709
AMH (pmol/L)		24.4 (21.1)	25.8 (30.1)	0.7117
Day 2/3 LH (IU)		1.2 (2.7)	2.1 (3.3)	0.0275
Day 2/3 FSH (IU)		6.9 (2.5)	6.4 (3.2)	0.5714
E2 on trigger (pmol/L)		13,445 (14,553)	12,603 (9,996)	0.5473
Total GT (IU)		4,088 (1,003)	3,894 (1,025)	0.2998
Maturation rate (%)[a]		72.0 (22.0)	76.0 (21.5)	0.2200
Fertilization rate (%)		82.5 (17.2)	83.0 (19.0)	0.7202
Blastocyst rate (%)[a]		59.5 (27.2)	50.0 (40.0)	0.5128
Euploidy rate (%)		67.0 (22.0)	60.0 (26.0)	0.0245

Normality was tested using the Shapiro–Wilk test.
AMH Anti-Müllerian Hormone, *IQR* Interquartile range, *THC* Tetrahydrocannabinol, *BMI* Body Mass Index, *LH* Luteinizing Hormone, *FSH* Follicle Stimulating Hormone, *E2* Estradiol, *GT* Gonadotropins.
[a]Indicates normally distributed data, all others are non-normally distributed. Significance was assessed using either a two-sided Mann–Whitney test or two-sided unpaired t-test, where appropriate.

Table 2 | Multiple logistic regression models for blastulation and euploidy rates

Outcomes	Covariates	Coefficient (β)	SE	p-value	OR	95% CI for OR	
						Lower	Upper
Blastulation rate >50%	THC+ (Case)	−0.803	0.338	0.018	0.448	0.229	0.864
	Oocyte age	−0.089	0.027	0.001	0.914	0.866	0.963
Euploidy rate >60%	THC+ (Case)	−0.759	0.365	0.038	0.468	0.226	0.952
	Oocyte age	−0.089	0.029	0.002	0.915	0.862	0.968

Significance was assessed using Likelihood Ratio Test (LRT)
SE Standard error, *CI* Coefficient intervals, *OR* Odds ratios, *THC* Tetrahydrocannabinol

physiological and supraphysiological concentrations, appears to accelerate oocyte maturation speed and completion, consistent with results from the López-Cardona bovine study[29] and previous mouse studies[26].

Oocytes must not only successfully progress through meiosis II to reach metaphase-II but also need sufficient time to reach cytoplasmic maturity to support early embryo development[30,41]. This process involves the precise and faithful packaging of various components, including maternal mRNA transcripts. mRNA production and storage in the ooplasm is critical, since, following establishment of the germinal vesicle, chromatin is condensed, and transcription is halted[43,44]. These transcripts are critical not only for meiosis resumption and fertilization, but also during the first 3 days of embryonic development[45], since the early embryo remains transcriptionally silent, relying entirely on maternally inherited mRNA from the ooplasm to drive cellular processes prior to embryonic genome activation[46]. The selection and enrichment of critical transcripts in the ooplasm have been described in the literature and are regulated by post-transcriptional mechanisms. These complex processes involve a sophisticated network of RNA binding proteins (RBPs), polyadenylation factors and RNA translational and degradation machinery, which all regulate the storage, translation, and degradation of oocyte mRNAs[46].

To understand how THC affects stored maternal mRNA during oocyte maturation, we identified genes associated with transcripts that were differentially expressed following THC exposure (Fig. 3a, c). Focusing on the common protein-coding transcripts, 32 genes were identified and grouped into nine principal functions: G protein-coupled receptor (GPCR) signaling, extracellular matrix regulation, embryogenesis, cell-cell communication, inflammation, cytoskeleton, detoxification, transcription factor and ion channels (Supplementary Data 5).

Among these, *MMP9* was significantly downregulated in both THC treatment groups. *MMP9* encodes for matrix metalloproteinase 9 (MMP-9) essential for local proteolysis of the extracellular matrix and leukocyte migration[47–49]. In animal models, MMP-9 has well-established role in ovulation and follicle rupture[50–52], and in mice, protein expression in blastocyst and early embryo is critical for implantation[53–58]. In humans, several studies have investigated its role in implantation using various trophoblast and implantation models[59–61]. Dysregulation of its expression and activity is associated with pregnancy complications and recurrent pregnancy loss[62–65]. Moreover, THC has also been shown to decrease MMP-9 expression in human amniotic epithelium[66] and endothelial cancer cells[67]. Thus, downregulation of *MMP9* in the ooplasm may negatively contribute to key ovulation events needed for fertilization, embryo development, and implantation.

G-protein coupled receptor (GPCR) signaling was also dysregulated following exposure to THC. GPCR signaling is crucial for oocyte growth and maturation, and many GPCRs are present at the oocyte surface, including the cannabinoid receptor 1 (CB1) and 2 (CB2)[27,68]. Genes coding for Regulators of G protein Signaling (RGS), *RGS5* and *RGS18*, were significantly upregulated following THC treatment in our study. These regulators are known modulate GPCR signal transduction[69]. *RGS5* and *RGS18* belong to the RGS R4 family and have been shown to bind with the Gα proteins, thus reducing their

inhibitory activity[70,71]. They have been shown to have a crucial role in the cell processing of the signal coming from the GPCRs[72]. Although the exact role of *RGS5* and *RGS18* in oocyte maturation is poorly understood, their upregulation suggests a potential impact on GPCR signaling pathways in response to THC stimulation.

Immune pathways were also overrepresented in both treatment groups compared to controls. For instance, *IFNG* and *IL33* were both significantly downregulated in THC exposed groups. It is well established that tight regulation of inflammatory processes is critical for implantation of the embryo in the endometrium[73]. Interferon-γ (IFNγ) is a cytokine widely known for its role in inflammation and the activation of macrophages[74]. Although its role during oocyte maturation is unclear, IFNγ secretion by the conceptus is essential for implantation in animal models[75]. On the other hand, Interleukin-33 (IL-33), a cytokine that belongs to the IL-1 family, binds to the ST2 (IL-1RL1) receptor[76]. In mice, IL-33 was found to be expressed in the oocyte and ST2 in granulosa cells and the uterus[77]. In humans, altered levels or expression of IL-33 or ST2 in various tissue and sample types were associated with pregnancy complications like preeclampsia[78–80], preterm birth[81–83], intrauterine growth restriction[84], and miscarriage[85]. The downregulation of IL-33 we observed in THC-exposed oocytes suggests there may be potential disruption in the inflammatory processes necessary for successful pregnancy.

A significant number of DEGs in THC-exposed oocytes are involved in the cytoskeletal function, including *KRT19, COL8A2, ACTA1* and *ARHGEF19* (Supplementary Data 5). In addition to these transcriptomic changes, we observed significant alterations to cytoskeleton machinery throughout this study. Indeed, the oocyte's cytoskeleton plays a crucial role in chromosome alignment, segregation, and polarity establishment[86] and without appropriate formation and regulation of key cytoskeletal functions, the oocyte is vulnerable to chromosomal abnormalities. However, the cytoskeleton-associated DEGs identified in this study have not been previously characterized in oocyte development and thus require further investigation to gain a deeper understanding of the effects of THC on these processes.

Taken together, THC exposure seems to impact critical transcripts involved in key oocyte maturation processes, fertilization, early embryo development and implantation. While these transcriptomic alterations likely result from post-transcriptional processes, the specific mechanisms by which THC affects these processes in human oocytes remains unknown[46].

Given the observed increase in nuclear maturation rate and the altered transcriptomic profiles related to chromosome organization, we next investigated the impact of THC on chromosome segregation. Indeed, errors in chromosome segregation during the first meiotic division is the most frequent cause of embryo aneuploidy[87], making the faithful establishment of chromosome segregation machinery a critical bottleneck in the production of a chromosomally normal embryo[88,89]. To investigate oocyte ploidy, we performed polar body biopsy and low-pass whole genome sequencing. Notably, we observed that THC exposure led to a 9% increase in aneuploidy rates (Fig. 4b). Additionally, we observed an increase in the proportion of oocytes with complex aneuploidies (defined as a gain or a loss of 3 or more chromosomes)[35] in the THC-treated group compared to controls (Fig. 4c). Aneuploidies are associated with implantation failure, miscarriage, and are incompatible with life[90]. It has been postulated that most aneuploidies arise from errors in maternal meiosis I[91–96], but our data suggest that meiosis II may also be sensitive to perturbations as 38% of the euploid oocytes had abnormal spindle morphology (Fig. 4d) determining using confocal microscopy. We assessed spindle morphology after 24 h incubation of oocytes with and without THC. A normal spindle configuration is barrel shaped with chromosomes aligned at the metaphase plate, while 'abnormal' configurations include multipolar spindles and misaligned chromosomes[97] (Fig. 4e). In this study, we demonstrated a dose-dependent decrease in the proportion of oocytes with normal spindle morphology following THC exposure (Fig. 4f). Correct chromosome segregation during oocyte maturation is essential for producing euploid embryos, which have the highest chance of establishing a healthy pregnancy[98].

To address the primary clinical question regarding THC's impact on IVF outcomes, we used a pairwise case-control matching strategy, where each positive sample was matched to two negative samples based on demographic data, and we compiled the resulting matched cohort's IVF outcomes (Table 1). THC exposure was associated with a marginal increase in maturation rate (Table 1), concordant with what was obtained in our in vitro experimentations (Fig. 2b). Further, a significant decrease in embryo euploidy rate (Table 1) and reduced odds of obtaining a euploidy rate above 60% was observed (Table 2). These results indicate that THC-positive patients may have fewer euploid embryos from their IVF cycle and may experience a longer time to pregnancy.

To deepen our understanding of our findings, we must extrapolate what is known about THC interactions and pathways from other cell types. THC primarily elicits its functions through binding the cannabinoid 1 and 2 receptors (CB1 and CB2), which are expressed at all stages of oocyte maturation[68]. CB1 and CB2 are G protein-coupled receptors (GPCRs) which are capable of inhibiting adenylate cyclase, the enzyme responsible for catalyzing adenosine triphosphate (ATP) to cyclic adenosine 3′, 5′-monophosphate (cAMP). Activation of the CB receptors, through stimulation by THC, could thus lead to an inhibition of adenylate cyclase, resulting in lowering ooplasm cAMP levels[99]. Adenylate cyclase activity and the constant and high production of cAMP is critical to prevent premature meiotic resumption[100]. Here, we propose a hypothetical model of action of THC wherein THC binds to CB1/2 activating them, which in turn inhibits adenylate cyclase activity, reducing ooplasm cAMP concentration. Releasing the inhibition of meiotic resumption, would then result in premature resumption of meiosis. This untimely and premature resumption may increase the likelihood of aneuploidy arising in the oocyte and resulting embryo due to the premature separation of chromosomes misaligned on the metaphase plate and an asymmetrical division of the chromosomes into the first polar body. This hypothesis aligns with the correlation between THC concentrations and oocyte maturation rate observed in the retrospective cohort (Fig. 1d) and the increased oocyte maturation rate in vitro, as well as the associated reduction in euploid oocytes (Fig. 4b) and euploid embryo rates (Table 1) we observed. Further investigations are underway to dive deeper into THC signaling in the oocyte and better refine this hypothetical model.

To conclude, this study comprehensively investigates and demonstrate the impact of THC on the human oocyte. Herein, our findings reveal significant effects on oocyte maturation, transcriptomic profiles, meiotic spindle organization, and oocyte ploidy. Collectively, this data presents compelling evidence that cannabis consumption may negatively impact female fertility. Our integrated and multi-faceted in vitro approach, utilizing multiple techniques and endpoints to assess chromosome segregation, is a major strength of this study. However, it was limited by the usage of immature GV oocytes following ovarian hyperstimulation, which are considered suboptimal for reproductive purposes, since they did not mature following initial stimulation. Furthermore, we acknowledge the importance of patient age on the oocyte ability to mature in vitro, but this study was not statistically powered to analyze results based on patient age. This limitation arose because the majority of GV oocytes were retrieved from patients younger than 37 years old (81%). Also, our study focused on identifying changes in the abundance of the prestored transcripts in response to THC exposure, rather than de novo transcription, limiting our ability to speculate on the impact of THC on gene expression before the GV stage.

On the other hand, our retrospective cohort objectively measured THC and its metabolites to determine the impact of THC on IVF

outcomes, overcoming biases inherent in self-reporting[101]. Indeed, 73% of our patients positive for THC did not report cannabis use when completing their patient intake questionnaire, potentially due to the persistent stigma of recreational drug consumption. A limitation of our retrospective study is the lack of data on cannabis consumption habits (e.g. frequency, timing, dosage, route of consumption), and our cohort is likely not representative of the general population, as all patients were undergoing IVF for fertility treatment or to altruistically donate their oocytes to intended parents. In addition, FF was not measured for the presence of other drugs, and even though none of the patients reported concomitant use of other drugs, self-reporting alone cannot rule out the exposure of the follicle to these substances. The limitations associated with the retrospective aspect of this study and the other potential contributors (e.g. lifestyle habits) to the observed outcomes are compensated by our in vitro study that used at least three oocytes (one in each exposure group) per patient.

Finally, these findings underscore the need for increased awareness and caution among people with ovaries, particularly those undergoing fertility treatments. Our study highlights the importance of informing patients about the potential risks associated with cannabis consumption and provides a basis for regulatory bodies, medical professional societies, and public health organizations to establish recommendations and guidelines regarding cannabis consumption during fertility treatment.

Methods

Ethical approval and cannabis regulatory licencing

All patients undergoing ART procedures were provided with the opportunity to participate in the collection, and future use, of biological waste material for research purposes. Patients were provided with an Independent Review Board (IRB) approved informed consent package containing information regarding the types of material that would be collected following consent as well as examples of projects this material may be used for. Patients did not receive compensation or financial benefit for their participation in the collection of biological waste material. All patients included in this study provided informed consent for the donation of their biological waste material, which included follicular fluid (FF) and immature (GV) oocytes as well as associated de-identified demographic and clinical information, including age, Body Mass Index (BMI), ovarian reserve metrics and treatment regimens (Veritas IRB Approval #16487). To be included in the assessment of tetrahydrocannabinol (THC) on in vitro maturation (IVM), patients must have had 10 or more MII oocytes after stripping and a minimum of 3 GV oocytes in order to have at least one GV oocyte per treatment group. Last, all patients included in the assessment of THC on IVM were confirmed to be negative for THC by LC-MS/MS (described below). Patients were excluded if: they did not meet inclusion criteria, had low oocyte yield (<16 oocytes), low oocyte maturation rate (<62.5%), a previous cycle with poor fertilization rate (<75%) and/or blastulation rate (<40%), severe male factor, advanced maternal age (>40 years old), or who were undergoing fertility preservation (oncofertility and/or social egg freezing). Moreover, if a patient was consented for the donation of their biological waste material but the physician and embryologist deemed rescue-IVM (rIVM) was indicated for their clinical care, no oocytes would be collected for research purposes and patients would be informed of the addition of rIVM to their clinical treatment by the physician or another healthcare professional. All GV oocytes included in this study were collected and underwent IVM between July 2022 and January 2024. The request and use of samples and de-identified demographic and clinical data for this study was approved by Veritas IRB Approval #16518. For the retrospective analysis, FF was collected from all consenting patients undergoing IVF treatment between June 2016 and March 2023. All samples in this study were considered "female" as they are human oocytes which are chromosomally "XX". Gender, race, ethnicity or other socially relevant groupings were not considered in this study. The purchase, storage and use of Δ9-THC and its metabolites for research purposes was approved by Health Canada and all procedures were conducted in accordance with the 'Cannabis Act' and 'Cannabis Regulations' (License #LIC-A4MUR820SB-2020).

Exocannabinoid detection in FF

Measurements of Δ9-THC, 11-OH-THC and 11-COOH-THC in FF were performed for every patient who donated GV oocytes, as previously described, to exclude patients consuming cannabis for the IVM investigation[23]. For the retrospective study, FF and matched serum were measured using the same procedure. Briefly, proteins were precipitated using methanol (1:1 v/v), and the supernatants were assessed by LC-MS/MS using a QTRAP 5500 (SCIEZ, Concord, CA, USA) and Agilent 1290 HPLC (Agilent, Santa Clara, USA) with a calibration curve (0.001–200 ng) of known amount of the molecules of interest. Samples above the lower limit of quantification were considered positive. Cannabinol (CBN) and Cannabidiol (CBD) were also measured in the samples but were undetectable.

Oocyte in vitro maturation

GV oocytes were received from consenting patients, randomly split into three groups, and cultured using standard clinical IVM media (SAGE One-step (CooperSurgical, Canada) + 7.5 IU/mL of Menopur (Ferring, Canada)): Ctrl ($n = 96$, only IVM media), THC1 ($n = 95$, treated with a physiological concentration of cannabis based on previous measurements of cannabis in FF[23]: 25 ng/mL Δ9-THC (Sigma-Aldrich, Canada), 5 ng/mL 11-OH-THC (Sigma-Aldrich, Canada), 50 ng/mL 11-COOH-THC (Sigma-Aldrich, Canada)) and THC2 ($n = 94$, treated with THC at a concentration based on previous animal studies[25,27,29]: 100 ng/mL Δ9-THC, 50 ng/mL 11-OH-THC, 200 ng/mL 11-COOH-THC). Oocytes were obtained from 24 patients, and their demographic data are reported in the Supplementary Table 1. Images using an inverted bright field microscope were taken prior and following incubation to assess oocyte morphology. Oocytes were cultured for 24 h using the EVOS FL Auto 2 (ThermoFisher, Canada) imaging system or cell culture incubator (5% CO_2 and 37 °C). Timelapse images were taken every 15 min and used for the assessment of GVBD and polar body extrusion (Ctrl: Supplementary video 1, THC1: Supplementary video 2 and THC2: Supplementary video 3). After 24 h of culture, oocytes were processed according to the specific endpoint (RNAseq or immunostaining/polar body biopsies).

Single oocyte mRNA sequencing

Oocytes destined for single-cell RNASeq were further stripped of any residual cumulus cells and snap-frozen in 0.2 mL tubes in less than 1 μL phosphate-buffered saline (1 X PBS). To reduce inter-sample variability, we selected patients with similar demographics and stimulation parameters (Supplementary Table 2). A total of 86 oocytes from 24 patients were selected for RNASeq, 28 in Ctrl, 27 in THC1 and 31 in THC2. cDNA from single oocytes were synthesized using the SMART-seq v4 Ultra Low Input RNA Kit (Takara Bio Inc., Japan). The amplified cDNA was purified using Agencourt AMPure XP beads (Beckman Coulter, USA) and eluted. The purified cDNA was quantified using the Qubit dsDNA high sensitivity assay (ThermoFisher, Canada) and length and molarity were assessed using the DNF-474 HS NGS Fragment Kit (1-6000 bp) with the Fragment Analyzer 5200 (Agilent Technologies, USA). The amplified cDNA (0.2 ng) was used to construct sequencing libraries using a modified and miniaturized Nextera XT library preparation protocol (Illumina, Canada) developed for the use with the Mosquito HV liquid handling robot (SPT labtech, Boston, USA). The quality of the libraries was assessed using the same Fragment Analyzer kit DNF-474 HS NGS Fragment Kit (1–6000 bp), quantified using Qubit dsDNA high sensitivity assay (ThermoFisher), normalized and pooled. Sequencing was performed on a NovaSeq 6000 S2 flow cell (Illumina, Canada) at

Princess Margaret Genomics Centre (2 × 150 bp) (Toronto, Canada). The sequencing quality control metrics are compiled in the Supplementary Data 6.

Single oocyte RNASeq bioinformatics

Raw sequencing reads were trimmed based on read quality (Phred > 28) and aligned and quantified to hg38 using STAR (Spliced Transcripts Alignment to a Reference; v2.7.8a)[102]. Low abundant transcripts were excluded (maximum <20) and normalized using the default normalization method built into DESeq2 (v3.5)[103]. We conducted differential expression (DE) using DESeq2 comparing THC1 vs. Ctrl and THC2 vs. Ctrl. Significantly differentially expressed genes were defined as p-value < 0.05 and $|\log_2$fold change (FC)| of >1. The complete list of DE genes is available in the Supplementary data 1 (THC1 vs Ctrl) and the Supplementary data 2 (THC2 vs Ctrl)). This analysis was conducted in Partek Flow (version 11.0.23.1004). Gene Set Enrichment Analysis (GSEA)[104,105] was conducted to determine what gene sets were impacted by exposure to THC. The resulting pathway list was cross referenced with a custom gene set created and supported by the Bader Lab (University of Toronto) which is comprised of all GO database, KEGG, Reactome, and Wiki pathways gene sets (v2024-01-01) (http://download.baderlab.org/EM_Genesets/)[106] (Supplementary Data 3) Significant pathways were defined as having a |Normalized Enrichment Score (NES)| > 1.5 and p-value < 0.05.

Immunostaining and imaging

After 24 h in culture, the zona pellucida (ZP) was removed by incubating (30 s–2 min) with EmbryoMax® Acidic Tyrode's Solution (Millipore Sigma, CA) and polar bodies were mechanically separated from the oocytes. Oocytes were immediately fixed with 3.7% paraformaldehyde in PHEM (PIPES 12 mM, HEPES 5 mM, EGTA 2 mM and MgSO4· 7H2O 0.8 mM, pH 6.9) for 30 min at room temperature (RT) and then permeabilized in PHEM + 0.25% Triton-X for 15 min at RT. After permeabilization, they were incubated overnight at 4 °C in blocking solution (3% bovine serum albumin (BSA) + 0.05% Tween-20 in 1X PBS). On the next day, the plate was brought to RT before transferring the oocytes in the primary antibody solution for 1 h at 37 °C (mouse anti-α-Tubulin (1:250), T6199, Sigma-Aldrich, USA). Then, the oocytes were washed three times in the wash solution (0.5% BSA + 0.05% Tween-20 in PBS) at RT and moved in the secondary antibody solution for 2 h at 37 °C (goat anti-mouse AlexaFluor 488 (1:200), A-11001, Invitrogen, USA and Phalloidin AlexaFluor 555 (1:500) (A34055, Invitrogen, USA). After secondary antibody solution, the oocytes were washed three times in the wash solution and transferred into Hoechst 33342 (1:500) for 30 min. Finally, the oocytes were transferred into 1.5 μL drops in an imaging dish (Nunc Glass Bottom Dish, ThermoScientific, CA) covered with paraffin oil and 0.2 μm z-stacks were captured using Leica SP8 confocal microscope using the 63× objective with oil and a zoom factor set at 8 at the Advanced Optical Microscopy Facility (Toronto, Canada). Deconvolution was applied on the images using Huygens software version 23.10 (https://svi.nl/Huygens-Software) and images were visualized using ImarisViewer 10.1.1. Negative controls consisted of GV oocytes to observe the absence of spindle structure and stained MII-oocytes without the mouse anti-α-Tubulin primary antibody (Supplementary Fig. 4).

PB biopsy, whole genome amplification, sequencing and analysis

Polar bodies were separated from the oocytes after removal of the ZP eliminating possible somatic cell contamination (as shown in Supplementary Fig. 3). Polar bodies were individually snap-frozen at −80 °C in less than 2 μL and blinded samples were sent for whole genome chromosome copy number variation assessment (CNV) to the CReATe Reproductive Genetics sequencing platform. CNV analysis was performed by low-pass whole genome Next Generation Sequencing using validated clinical workflow on Illumina platform. Briefly, gDNA was amplified using SurePlex Whole Genome Amplification (WGA) (Illumina, CA), according to manufacturer's instructions. Amplified gDNA was then tagmented and indexed using Nextera XT (Illumina, CA). The indexed libraries were purified using AMPure XP beads (1:1 ratio) and normalized using magnetic beads. The normalized libraries were pooled, denatured, and sequenced on a NextSeq 550 (paired end, 2 × 75 bp). NxClinical version 6.0 (Bionano, CA) was used for chromosome CNV analysis and data visualization according to our standard clinical procedure (2 million reads/sample, CNV resolution of >10 Mb). Optimization experimentations demonstrated the concordance between polar body chromosome numbers and its sister oocyte (Supplementary Fig. 3).

Statistical analysis

Datasets were first assessed for normality using the Shapiro–Wilk test. Statistical significance was determined using two-sided Fisher's exact test for contingency analysis (maturation rate, spindle morphology and euploid rates), One-way ANOVA with a two-sided Holm–Sidak's multiple comparison test for continuous normally distributed datasets (oocyte diameter), or Kruskal–Wallis with a two-sided Dunn's multiple comparison test for continuous non-normally distributed datasets. The specific statistical test is indicated throughout table and figure legends. Significance was defined as $p < 0.05$. For the retrospective analysis, we performed pairwise case-control matching, where each THC-positive sample was matched to two THC-negative samples, as determined by the presence/absence of 11-COOH-THC in the FF. Matching was conducted using the FUZZY matching command in Statistical Package for Social Sciences (SPSS-v29) based on the following covariates: oocyte age, participant body mass index (BMI), anti-müllerian hormone (AMH), day 2/3 luteinizing hormone (LH), and follicle stimulating hormone (FSH), (estradiol) E2 on trigger, and total gonadotropin (GT) dose. AMH, LH, FSH and E2 were quantified during the clinical assessment using the Cobas e411 instrument (Roche, Basel, Switzerland). Matching success was determined using a two-tailed Mann–Whitney test for non-parametric distribution. Statistical significance of the IVF outcomes was determined using two-tailed Mann–Whitney test for non-parametric distribution (fertilization and euploid rates) and unpaired two-tailed t-test for parametric distribution (maturation and blastocyst rates) using GraphPad Prism 10.2.3. Numbers in parentheses in each figure legends represent distinct samples and not repeated measures.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Data generated in this study are provided in the Supplementary Information/Source Data file. The normalized read count expression matrix and metadata have been deposited in the Gene Expression Omnibus (GEO) database with accession number GSE297757. Due to participant privacy concerns and institutional ethics restrictions, raw sequencing files (fastq) have been deposited at the European Genome-phenome Archive (EGA), which is hosted by the European Bioinformatics Institute (EBI) and the Centre for Genomic Regulation (CRG), under accession number EGAS50000001052. Further information about EGA can be found at https://ega-archive.org. Expected timeframe for response to access requests is 10 business days and the data will be available for the duration of the study as defined by the Data Access Agreement associated with this dataset. Source data are provided with this paper.

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Author contributions

C.D., B.W. and N.F.W. participated in the design of the study. C.D. and B.W. established the collection of immature human oocytes through the CReATe Biobank in collaboration with IK and her team helping coordinate the oocyte collection. C.D. received, cultured and froze the oocytes for all experiments. C.D. optimized and conducted oocyte staining and confocal imaging, polar body biopsies, and RNA sequencing related manipulations. C.D. and B.W. performed the transcriptomic analysis. C.D. and B.W. compiled the retrospective data and analyzed the results. S.M. and her team conducted the polar body sequencing to assess the ploidy status. C.D. and B.W. wrote the manuscript. C.D. hand-drawn all graphical elements. C.L. provided critical feedback on study design and the manuscript. All authors provided feedback on the manuscript and read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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