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REVIEW

Biomarkers Assessing the Role of Cumulus Cells on IVF Outcomes: A Systematic Review

Gaelle Massoud[1] · Marcus Spann[2] · Kamaria Cayton Vaught[1] · Samarjit Das[3] · Mark Dow[4] · Richard Cochran[1] · Valerie Baker[4] · James Segars[1] · Bhuchitra Singh[1]

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Abstract

Purpose Although significant improvements in assisted reproductive technology (ART) outcomes have been accomplished, a critical question remains: which embryo is most likely to result in a pregnancy? Embryo selection is currently based on morphological and genetic criteria; however, these criteria do not fully predict good-quality embryos and additional objective criteria are needed. The cumulus cells are critical for oocyte and embryo development. This systematic review assessed biomarkers in cumulus-oocyte complexes and their association with successful IVF outcomes.

Methods A comprehensive search was conducted using PubMed, Embase, Scopus, and Web of Science from inception until November 2022. Only English-language publications were included. Inclusion criteria consisted of papers that evaluated *genetic biomarkers* associated with the cumulus cells (CCs) in humans and the following three outcomes of interest: oocyte quality, embryo quality, and clinical outcomes, including fertilization, implantation, pregnancy, and live birth rates.

Results The search revealed 446 studies of which 42 met eligibility criteria. Nineteen studies correlated genetic and biochemical biomarkers in CCs with oocyte quality. A positive correlation was reported between oocyte quality and increased mRNA expression in CCs of genes encoding for calcium homeostasis (*CAMK1D*), glucose metabolism (*PFKP*), extracellular matrix (*HAS2*, *VCAN*), TGF-β family (*GDF9*, *BMP15*), and prostaglandin synthesis (*PTGS2*). Nineteen studies correlated genetic and biochemical biomarkers in CCs with embryo quality. A positive correlation was reported between embryo quality and increased mRNA expression in CCs of genes encoding for extracellular matrix (*HAS2*), prostaglandin synthesis (*PTGS2*), steroidogenesis (*GREM1*), and decreased expression of gene encoding for hormone receptor (*AMHR2*). Twenty-two studies assessed genetic and biochemical biomarkers in CCs with clinical outcomes. Increased expression of genes encoding for extracellular matrix (*VCAN*), and TGF-β family (*GDF9*, *BMP15*) were positively correlated with pregnancy rate.

Conclusion Genetic biomarkers from cumulus cells were associated with oocyte quality (*CAMK1D*, *PFKP*, *HAS2*, *VCAN*, *GDF-9*, *BMP-15*, *PTGS2*), embryo quality (*GREM1*, *PTGS2*, *HAS2*), and pregnancy rate (*GDF9*, *BMP15*, *VCAN*). These results might help guide future studies directed at tests of cumulus cells to devise objective criteria to predict IVF outcomes.

Keywords Cumulus-oocyte complex · Cumulus cells · Biomarkers · Oocyte quality · Embryo quality · IVF outcomes

✉ Bhuchitra Singh
bsingh10@jhmi.edu

[1] Division of Reproductive Sciences & Women's Health Research, Department of Gynecology & Obstetrics, Johns Hopkins University School of Medicine, 720 Rutland Avenue, Ross Research Building, Room 624, Baltimore, MD 21205, USA

[2] Informationist Services, Welch Medical Library, Johns Hopkins University School of Medicine, Baltimore, MD, USA

[3] Department of Pathology and Critical Care Medicine, Johns Hopkins School of Medicine, Baltimore, MD, USA

[4] Division of Reproductive Endocrinology and Infertility, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction

Embryo selection yielding successful in vitro fertilization (IVF) outcomes remains one of the major challenges of assisted reproductive technology (ART). To date, embryo selection is based on morphological criteria and chromosomal status [1, 2]. Preimplantation genetic testing (PGT) has greatly improved the prediction of IVF outcomes, however, the use of cumulus cells (CCs) biomarkers could complement current technologies and further improve IVF outcomes.

The use of cells around the oocytes to determine oocyte and embryo quality has been on the rise. Granulosa cells around oocytes differentiate into two different phenotypes during follicular development: the cumulus cells (CCs) surrounding the oocyte and the mural cells lining the follicular antrum. While the latter is important for estrogen production, cumulus cells are essential for oocyte development. Luteinizing hormone (LH) and follicle-stimulation hormone (FSH) surges lead to increased cAMP and cGMP as well as extracellular matrix production in CCs which results in their expansion to assist oocyte meiotic resumption [3]. Communication between CCs and oocytes is established via gap junctions which connect the cytoplasm of the oocytes to the CCs. Gap junctions between the CCs and oocytes are present on specific structures which are known as thin cytoplasmic projections called transzonal projections (TZPs) [3]. The bi-directional communication between the CCs and the oocytes through gap junctions is fundamental to the regulation of oocyte maturation by allowing the passage of cyclic nucleotides into the oocyte from the CC. cAMP and cGMP regulate oocyte maturation by preventing spontaneous meiotic activation from the germinal vesicle before the ovulatory signal and by enabling reinduction of meiosis after the ovulatory signal. In turn, oocytes communicate with the CCs via oocyte-secreted factors (OSFs) to dictate cumulus cell differentiation and support their growth and maturation. CCs, being in close proximity and communicating with oocytes, may therefore reflect oocyte function, and quality, as well as embryo developmental potential. Understanding the role of CCs during follicular development may help predict IVF outcomes including oocyte quality, embryo quality, chromosomal status, and clinical outcomes. With the emergence of new technologies for genotyping and gene expression analysis such as microarray, next generation sequencing (NGS), and reverse transcription-polymerase chain reaction (RT-PCR), many biomarkers have been identified and studied in the CCs to help assess for oocyte and embryo quality [4]. These biomarkers show promise in IVF success.

This systematic review evaluates the biomarkers from cumulus cells that assess oocyte quality, embryo quality, and IVF clinical outcomes such as fertilization, implantation, pregnancy, and live birth rates.

Methods

Study eligibility

Pre-clinical and observational studies reporting the role of biomarkers of CCs in IVF outcomes in English language were included. The IVF outcomes include oocyte quality, embryo quality, and clinical outcomes such as fertilization, implantation, pregnancy, and live birth rates. Review articles, abstracts, unpublished, and animal studies were excluded.

Patient selection criteria

Adults more than 18 years of age undergoing in vitro fertilization (IVF) treatment for unexplained infertility were included.

Types of outcomes measures

Primary outcomes included oocyte quality, embryo quality, and clinical outcomes such as fertilization, implantation, pregnancy, and live birth rates. Studies reporting one or more of the primary outcomes were included.

Search strategy and data sources

The search involved the following databases: PubMed, Embase, Cochrane, Web of Science, and Scopus, up until November 2022. The search strategy is detailed in Supplemental Item 1. No organizations or individuals working in the infertility field were contacted. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting guidelines were followed [5].

Data extraction and management

Title and abstract screening were completed first. All articles meeting inclusion criteria were included, and the full article was reviewed. Eligibility was assessed based on the information provided by the article. Data was extracted in a standardized sheet that included participant characteristics and outcomes of interest.

The risk of bias in observational studies was assessed using the Newcastle-Ottawa Scale (NOS). It was assessed as

good, fair, or poor. Quality was evaluated by the assessment of selection, comparability, and outcome categories.

Data was summarized narratively. The role of cumulus cell biomarkers in IVF outcomes was reported.

Results

Search results

The initial search yielded 446 potentially relevant studies that were identified. A total of 48 duplicates were removed resulting in 398 studies for title and abstract screening. Then, 240 studies were excluded. Overall, 157 articles were retrieved for full-text screening of which 42 met inclusion criteria (Fig. 1). Forty-two studies were included and published between 2005 and 2022. Of which, 4 were pre-clinical and 38 were observational studies (Table 1). Table 1 includes information on population and CCs sample size, type of study evaluated, and methods used to assess outcomes of interests. Oocyte quality was reported in 19 studies, embryo quality was reported in 19 studies, and clinical outcomes were reported in 22 studies. A total of 1959 individuals participated in the included studies. They were all adults between the ages of 22 and 45. They were diagnosed with infertility, and all were seeking IVF treatment. The quality of the included studies was assessed by the Newcastle Ottawa Scale and was found to be overall fair. Three studies were of good quality whereas 31 were of fair quality (Table 1).

Outcomes

Genetic biomarkers from cumulus cells and oocyte quality

Several studies examined the role of genetic biomarkers in CCs in assessing oocyte quality. Table 2 summarizes the association between CC biomarkers and oocyte quality. Nineteen studies investigated the association between genetic biomarkers of CCs and oocyte quality. Three out of 30 genetic biomarkers were examined by two or more studies. These three biomarkers include genes encoding for extracellular matrix (*VCAN*, *HAS2*) and genes encoding for prostaglandin synthesis *(PTGS2).* Ekart et al., Shen et al., and Wathlet et al. looked at the association between *VCAN* and oocyte quality, and they all demonstrated that decreased expression of *VCAN* was significantly associated with mature and competent oocytes ($p < 0.0001$; $p = 0.024$; $p < 0.05$, respectively) [20, 39, 43]. Ekart et al. and Scarica et al. looked at the association between *HAS2* and oocyte quality [20, 38]. These studies showed contradictory results. Both of them evaluated individual CC masses, the discrepancy in results might stem from the use of different housekeeping genes as controls for RT-PCR (*B2M*, *UBC* genes vs *RPL19* gene, respectively) [20, 38]. Additionally, Anderson et al., Scarica et al., and Wathlet et al. evaluated the association between *PTGS2* and oocyte quality. They all found that increased expression of *PTGS2* in CCs was significantly associated with competent and mature oocytes ($p = 0.05$) [7, 38, 43]. Anderson et al. found that a 4.7-fold increased *PTGS2* expression was significantly associated with oocyte maturity ($p = 0.001$) [7].

A correlation between biomarkers and oocyte quality has been established in several studies. Ito et al. found that *GSTT1*, the gene encoding for Glutathione S-Transferases, was negatively correlated with oocyte maturity ($r = -0.31$, $P < 0.05$) [26]. Li et al. showed that *BMP-15* and *GDF-9* were positively correlated with oocyte maturity [30], with a partial correlation coefficient of 0.345 ($p < 0.001$) and 0.353 ($p < 0.001$), respectively [30]. Scarica et al. found that *CAMK1D*, *a* gene responsible for calcium regulation, was a strong parameter associated with oocytes' developmental potential with an area under the curve (AUC) of 0.78 (95% CI 0.67–0.89, $p < 0.01$) [38]. Shen et al. used multivariate logistic regression to assess the correlation between *PFKP*, *a* gene encoding for glucose metabolism, and oocyte maturity. They found a positive correlation between *PFKP* and oocyte maturity ($p = 0.014$) [39].

Genetic biomarkers from cumulus cells and embryo quality

Several studies examined the role of genetic biomarkers in CCs in assessing embryo quality. Table 3 summarizes the association between CCs and embryo quality. Nineteen studies investigated the association between genetic biomarkers of CCs and embryo quality. Three out of 31 biomarkers were examined by two or more studies. These biomarkers include genes involved in follicular development (*GREM1*) and genes encoding for prostaglandin synthesis (*PTGS2*) as well as extracellular matrix (*VCAN*). Anderson et al., Cillo et al., and McKenzie et al. looked at the association between *GREM1* and embryo quality, and they all showed that increased expression of *GREM1* was associated with good-quality embryos [7, 14, 33]. McKenzie et al. further studied the predictive value of *GREM1* for embryo quality. It was shown to be a predictor of embryo quality with area under the curve (AUC) 0.81 (95% CI 0.71–0.90) [33]. They also found that a 5.2-fold increased relative expression of *GREM1* yields sensitivity and specificity for embryo quality of 83% and 81%, respectively [33]. Although the association evaluated by Anderson et al. between *GREM1* and embryo quality was not significant ($p = 0.09$), they found a significant positive predictive ability of embryo quality with AUC 0.61 (95% CI 0.53–0.70, $p = 0.012$). Anderson et al. and McKenzie et al. looked at the association between *PTGS2* and embryo quality. They both found that

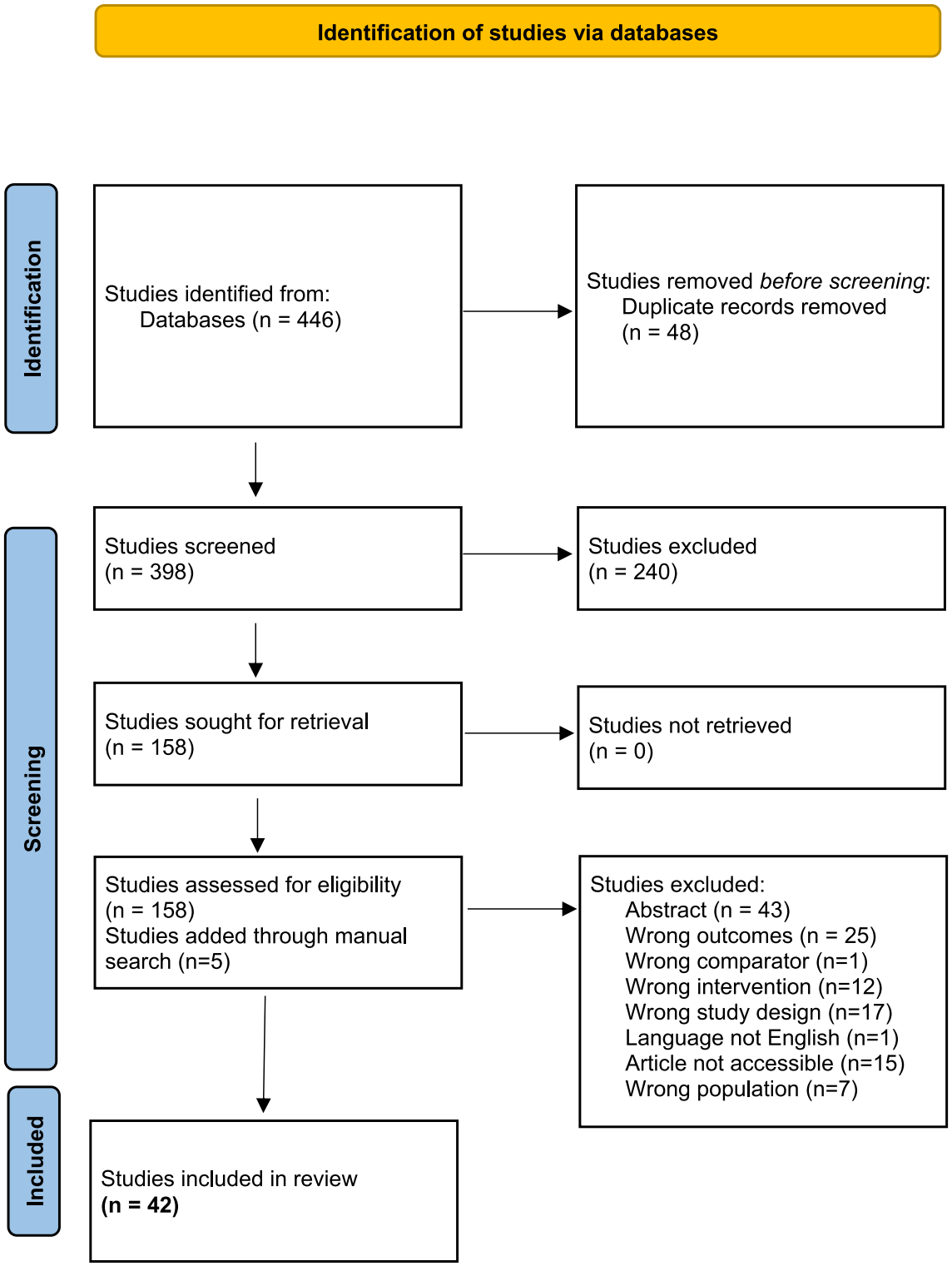

Fig. 1 PRISMA identification of studies flowchart

increased expression of *PTGS2* in CCs was significantly associated with good-quality embryos ($p < 0.05$) [7, 43]. McKenzie et al. also showed that *PTGS2* could be a predictor of embryo quality and that combining *PTGS2* and *GREM1* would improve the predictive value for embryo development compared to *GREM1* alone (AUC 0.82 vs 0.81, respectively), however, results were not significant [33]. Two studies examined the association between *VCAN*

Table 1 Summary of including studies assessing cumulus cell biomarkers and IVF outcomes

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Alfaidy et al. [6] •	Both ovaries were present and exempt from morphological abnormalities or surgical history; FSH < 10 IU/L on the third day of the unstimulated cycles; no current or past disease affecting the ovaries	Tobacco use, PCOS, recurrent pregnancy loss, or ectopic pregnancy, ovarian hyperstimulation syndrome, women with empty follicle syndrome	72 couples undergoing IVF with ICSI	ELISA assay on 52 CC samples Individual CCs	FSH or human menopausal gonadotropin following down-regulation with gonadotropin-releasing hormone agonists or antagonists	Increased expression of PROK1 in mature oocytes ($p < 0.01$)	Prospective cohort study Fair*
Anderson et al. [7] •	Women undergoing IVF with ICSI for male factor infertility, age average 35.2	_	75 women undergoing IVF with ICSI	RT-PCR on 674 oocytes Individual CCs Endogenous control for RT-PCR *GAPDH*	GnRH agonist long protocol	*I*ncreased mRNA expression of *PTGS2* (4.7-fold) was significantly associated with better oocyte quality ($p = 0.001$)	Prospective cohort study Fair*
Assidi et al. [8] •▲	Women undergoing IVF with ICSI	_	8 patients undergoing IVF with ICSI	Microarray, RT-PCR on two groups: Group 1: CCs of the zona good oocytes with successful pregnancy (ZGPs) Group 2: CCs of the zona good score with unsuccessful pregnancy (ZGNPs) Individual CCs Endogenous control for *ACTB*, *PPIA*	GnRH agonist long protocol	Increased expression of *DPP8* ($p = 0.0441$), *HIST1H4C* ($p = 0.0482$), *UBQLN1* ($p = 0.0236$), *CALM1*($p = 0.05$), *NRP1* ($p = 0.0107$), *and PSMD6* ($p = 0.0412$) was significantly associated with increased pregnancy rate Decreased expression of *TOM1* ($p = 0.0126$) was significantly associated with good oocyte quality	Prospective cohort study Fair*
Assou et al. [9] ▲	Patients undergoing IVF with ICSI for male infertility factor, age 30.9 ± 2.5	_	30 patients undergoing IVF with ICSI	Microarrays and RT-PCR on 50 CC samples Individual CCs Endogenous control for *PGK-1*	GnRH agonist or antagonist with recombinant FSH or with hMG	Increased expression of *BCL2L11* ($p = 0.25$), *PCK1* ($p = 0.22$) and decreased expression of *NFIB* ($p = 0.16$) was not significantly associated with successful pregnancy	Retrospective cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Baratas et al. [10] ●■	Women with unexplained infertility, age 33.5 ± 1.3, first IVF cycle	Obese, smoker, alcohol dependence, hypertension, diabetes, recent bacterial infection, genetically determined alterations, evidence of hydrosalpinx, or polycystic ovaries	25 couples undergoing IVF with ICSI	Chromatin dispersion test on 130 CC samples Individual CCs	Recombinant FSH with hMG followed by the administration of synthetic gonadotropin	Oocyte quality was negatively correlated with CCDFI ($p < 0.001$) Embryo development was negatively correlated with CCDFI ($p < 0.001$)	Cohort study Fair*
Bartolucci et al. [11] ●	Women undergoing IVF with ICSI and PGT-A, average age of 39 years old	Donated or thawed oocytes or surgically retrieved sperm	25 patients undergoing IVF with ICSI and PGT-A	PCR on CC samples Individual CCs Endogenous control for *NU6-2* and *RPL13a*	GnRH antagonist, leuprolide acetate down-regulation, and microflare lupron protocols	Increased immature and mature forms of *MIR-21-5p* in CCs of oocytes that developed into blastocysts compared to cumulus cells of oocytes that arrested prior to blastocyst formation ($p < 0.05$)	Cohort study Fair*
Bosco et al. [12] ■	Women undergoing IVF, FSH < 12 IU/ml, < 28 BMI, age < 38 years old	Azoospermia or severe oligoasthenospermic patients	53 women undergoing IVF	TUNEL and immunofluorescence assays on CC samples	GnRH agonist long protocol	DFI was higher in arrested vs transferred embryos ($p = 0.004$) pAKT/DFI ratio was higher in CCs of oocytes able to produce blastocysts, indicating that DFI is significantly lower when pAKT is higher	Prospective cohort study Fair*
Cheng et al. [13] ●■	Women undergoing IVF	Women with PCOS, endometriosis, and ovarian failure	80 women undergoing IVF	RT-PCR on 350 CC samples Individual CCs Endogenous control for *36B4*	GnRH agonist long protocol	Increased telomere length in CCs is associated with mature oocytes and good-quality embryos ($p < 0.05$)	Prospective cohort study Fair*
Cillo et al. [14] ■▲	Women undergoing IVF, average age of 36.5 years	_	45 women undergoing IVF	RT-PCR on 90 CC samples Individual CCs Endogenous control for *28S rRNA*	GnRH agonists or antagonists	Increased HAS2 expression in good-quality embryos compared to oocytes that did not fertilize or poor-quality embryos ($p < 0.05$)	Retrospective cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Daei-Farshbaf et al. [15] ■▲	Women undergoing IVF with ICSI	History of Cushing's syndrome, diabetes mellitus, congenital adrenal hyperplasia, PCOS, endometriosis, and those > 36 years	22 women undergoing IVF with ICSI	RT-PCR and Western Blotting on CC samples Individual CCs Endogenous control for *GAPDH*	GnRH agonist	Increased expression of OR10H2 was associated with high-quality embryo and fertilization rate ($p < 0.05$)	Cohort study
Daei-Farshbaf et al. [16] ●	Women undergoing IVF with ICSI	BMI > 26 kg/m2, history of Cushing's syndrome, diabetes mellitus, endometriosis, PCOS, congenital adrenal hyperplasia, androgen-secreting tumors	20 women undergoing IVF with ICSI	RT-PCR and Western blotting on CC samples Endogenous control for *GAPDH*	GnRH agonist	Increased expression of PPP3CB in CCs of MII oocytes compared with those in the GV, MI and unfertilized MII oocytes ($p \leq 0.05$)	Laboratory study
Demiray et al. [17] ●■	Women undergoing IVF, aged 25–33 years	_	9 women undergoing IVF	Immunofluorescence on 60 CC samples Individual CCs	Recombinant FSH with GnRH agonist	Increased levels of BMP2 were associated with better oocyte and embryo quality No significant differences were observed for AMH or CD90/THY1	Prospective case study
Desquiret-dumas [18] ■▲	Patients undergoing IVF with ICSI	_	62 patients undergoing IVF with ICSI	RT-PCR on 452 CGC samples Individual CCs	GnRH antagonists in combination with recombinant follicle-stimulating hormone (rFSH)	No link was found between oocyte fertilization and the CGC mtDNA content Embryo quality was associated with a greater quantity of CGC mtDNA ($p = 0.006$)	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Devjak et al. [19] ■	Patients undergoing classical IVF, < 35 years old, BMI between 17 and 26 kg/m2, tubal factor infertility	_	17 patients undergoing IVF	RT-PCR on 55 CC samples Individual CCs Endogenous control for RT-PCR *PPIB, 18s rRNA*	GnRH agonists or antagonists in combination with recombinant follicle-stimulating hormone (rFSH)	Increased mRNA expression of *AMHR2* was significantly associated with high-quality embryos ($p = 0.030$) In CCs with increased AMHR2 levels, high levels of LIF were further associated with better-quality embryos ($p = 0.033$)	Prospective cohort study Fair*
Ekart et al. [20] ●	Women undergoing IVF with ICSI	-	25 women undergoing IVF with ICSI	RT-PCR on 270 CC samples Individual CCs Endogenous control for *RPL19*	GnRH agonist	Decreased *VCAN* and *HAS2* expression was associated with mature oocytes	Retrospective study Fair*
Fang et al. [21] ■▲	Women undergoing IVF	_	103 women undergoing IVF with 32 ICSI	Western Blotting, RT-PCR on CC samples Endogenous control for *B-Actin*	_	Low Cited2 levels in CCs were associated with better embryo quality ($p < 0.05$), implantation rate ($p < 0.05$), and successful pregnancy ($p < 0.05$)	Retrospective cohort study Fair*
Feuerstein et al. [22] ▲	Women undergoing IVF, with ICSI average age was 33 years	_	106 women undergoing IVF with ICSI	Microarray and RT-PCR on CC samples Individual CCs Endogenous control for *NTC*	_	Increased expression of *RSG2* is associated with a higher pregnancy rate ($p < 0.05$)	Cohort study Good*
Gebhardt et al. [23] ▲	Patients undergoing SET	_	38 patients undergoing SET	RT-PCR on 38 CC samples Individual CCs Endogenous control for *GAPDH*	GnRH agonist long protocol	Increased expression of *VCAN* and *PTGS2* in CCs from oocytes that achieved term birth compared with those that failed to result in a successful pregnancy Increased expression of PTX3 in CCs from oocytes that resulted in live birth	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Hammond et al. [24] ■	Women undergoing IVF with ICSI	Women with PCOS or endometriosis	22 couples undergoing IVF	RT-PCR on CC samples Individual CCs TaqMan array human endogenous control panel	GnRH antagonist or microdose flare protocol or long protocol	Increased expression of *ATP51* was associated with good-quality embryo *($p < 0.05$)* Decreased expression of *HSPA5, PRDX3, VCAN* was associated with good-quality embryo *($p < 0.05$)*	Cohort study Fair*
Hasegawa et al. [25] •	Women undergoing IVF, age between 28 and 44, the indications for IVF included tubal absence or occlusion, ovulation disorders, endometriosis, oligozoospermia, and idiopathic infertility	_	44 women undergoing infertility treatment	RT-PCR and immunohistochemistry on 135 CC samples Individual CCs Endogenous control for RT-PCR *GAPDH*	GnRH agonist long protocol	Decreased expression of PR is associated with good oocyte morphology ($p < 0.05$)	Prospective cohort study Fair*
Ito et al. [26]	Women undergoing IVF	_	176 women undergoing IVF	RT-PCR on CC samples Endogenous control for *G3PDH*	_	Increased expression of GSTT1 in CGC was negatively correlated with oocyte maturity and development capacity ($p < 0.05$)	Laboratory study
Kim et al. [27] ▲	Women undergoing IVF	Women with infertility due to endocrine diseases, such as polycystic ovarian syndrome (PCOS) or women with genetic diseases were excluded.	24 women undergoing IVF	NGS, RT-PCR on CC samples Endogenous control for *B-Actin*	GnRH antagonist	Increased expression of LDLR and StAR and decreased expression of ADCY and HSD17B were associated with increased pregnancy rate ($P < 0.05$)	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Kordus et al. [28] ●■▲	Women undergoing IVF	Patients with endometriosis and DOR	15 women undergoing infertility treatment with PGT-A	RT-PCR on 163 CC samples Individual CCs Endogenous control for RT-PCR *TBP*	GnRH antagonist protocol	Increased mRNA expression of *PAPPA* was significantly associated with higher oocyte maturity (OR = 4.591, 95% CI 1.098 to 19.201) Decreased mRNA levels of *HSD3B*1 were significantly associated with oocytes that resulted in euploid embryos ($p < 0.05$) Decreased mRNA levels of *AREG* were significantly associated with higher live birth rate ($p < 0.05$) Increased mRNA expression of *LHGCR* was significantly associated with live birth rate ($p < 0.05$)	Prospective cohort pilot study Fair*
Lee et al. [29] ■	Women undergoing IVF	_	38 patients undergoing IVF	RT-PCR on 24 CC samples Endogenous control for *B-Actin*	Long protocol	Increased expression of *CKB* and *PRDX2* was associated with good-quality embryos in women > 38 years old In good-quality embryos, *CKB* expression was higher in the cumulus cells acquired from both older and younger age groups than in poor-quality embryos	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Li et al. [30] ■▲	Women undergoing IVF with ICSI, age ≤ 45 years, BMI of 17–35 kg/m^2	History of previous poor response, recurrent implantation failure (failed to achieve a pregnancy after three or more cycles), submucosal fibroids, intrauterine adhesion, congenital uterine malformation, hydrosalpinx, ovarian endometriomas > 3 cm in diameter, and PCOS	196 women undergoing IVF with ICSI	RT-PCR on 2426 CC samples Pooled CCs Endogenous control for *GAPDH*	Long protocol	Increased expression of *GDF9* and *BMP15* was associated with high-quality embryos ($p < 0.05$) Increased expression of *GDF9* and *BMP15* was associated with increased pregnancy rate ($p < 0.05$)	Retrospective cohort study Good*
Li et al. [31] ●▲	Women undergoing IVF with ICSI, younger than 38	_	40 women undergoing IVF with ICSI	RT-PCR on 308 CC samples Individual CCs Endogenous control for RT-PCR *RPL19*	GnRH antagonist protocol	Decreased expression of *GJA1* ($p = 0.0034$) and *SERPINE2* ($p = 0.0002$) was significantly associated with better oocyte quality Increased expression of *PRSS35* ($p = 0.0188$) was associated with increased fertilization rate Expression of *GJA1*, *SERPINE2*, *PRSS35*, and *PTX3* is not correlated with embryo quality	Prospective cohort study Fair*
Matos et al. [32] ▲	Women undergoing IVF with ICSI	_	_	Immunodetection, Western Blotting of CuZnSOD or MnSOD, and SOD activity quantification assay Endogenous control for *B-Actin*	_	Increased SOD activity level was associated with successful pregnancies ($p = 0.029$)	Cohort study Fair*
McKenzie et al. [33] ■	Women undergoing IVF with ICSI	Recent cigarette use and BMI > 25 kg/m^2	8 women undergoing IVF with ICSI	RT-PCR on 108 CC samples Endogenous control for *18S rRNA*	GnRH agonist	Increased *PTGS2, HAS2,* and *GREM1* expression was associated with higher-quality embryos	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Montazeri et al. [34] •	Women undergoing IVF, ≤ 37 years old	Women with any history of IVF failure, IVF cycles with donor gametes, advanced maternal age, severe endocrine conditions, recurrent pregnancy loss, and genetic disorders	100 infertile women undergoing IVF	RT-PCR on CC samples Endogenous control for RT-PCR *18s rRNA*	GnRH antagonist protocol	Increased expression of *AMHR2* and *FSHR* genes in CCs from immature compared to mature oocytes (4- to 6-fold) ($p < 0.0001$)	Prospective randomized study Fair*
Papamentzelopoulou et al. [35] ▲	Women undergoing IVF with ICSI, premenopausal, 25–45 years of age	_	40 patients undergoing IVF with ICSI	RT-PCR on CC samples Endogenous control for *G6PDH*	GnRH agonist long protocol	Increased *LHR* expression was not significantly associated with increased pregnancy rate	Cohort study Fair*
Papler et al. [36] ▲	Women undergoing IVF, < 35 years of age, BMI between 17 and 26 kg/m^2, tubal and unexplained cause of infertility, first or second IVF cycle, and normal partner's spermiogram	_	43 women undergoing IVF	RT-PCR on 43 CC samples Individual CCs Endogenous control for *GAPDH*	Recombinant FSH with GnRH antagonist	Expression levels EFNB2, *RGS2,* and *VCAN* were not associated with pregnancy	Cohort study
Papler et al. [37] ▲	Women undergoing IVF, age <35 years, BMI between 17 and 26 kg/m^2, tubal and unexplained cause of infertility, first or second IVF cycle, and normal partner's spermiogram	_	41 women undergoing IVF	RT-PCR on 55 CC samples Individual CCs Endogenous control for *GAPDH*	Short GnRH antagonist protocol	No significant gene expression differences in GC and CC surrounding fertilized and non-fertilized oocytes No significant gene expression differences between embryos that did or did not successfully implant	Cohort study

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Scarica et al. [38] ●	Women undergoing IVF with ICSI, average age 37.3 ± 4.0 years; average BMI 22.2 ± 3.6	Endometriosis and premature ovarian failure	18 patients undergoing IVF with ICSI	RT-PCR on 75 CC samples Individual CCs Endogenous control for *B2M* and *UBC*	GnRH antagonist	Increased expression of *CAMK1D* ($p = 0.01$), *PTGS2* ($p = 0.05$) was significant in oocytes reaching the blastocyst stage No statistically significant difference was instead found in the expression levels of *STC1* and *EFNB2* between oocytes that developed into blastocysts and arrested ones	Exploratory study
Shen et al. [39] ●■	Women undergoing IVF, age between 22 and 42 years, BMI was between 17.2 and 37.1 kg/m^2 basal FSH ranged from 3.46 to 13.62 IU/L	Endometriosis, abnormal chromosome	48 women undergoing IVF	RT-PCR on 354 CC samples Individual CCs Endogenous control for *B-Actin*	GnRH agonist	Increased expression of *PFKP* was associated with mature oocytes [OR = 1.07 (1.01, 1.12)] Increased expression of *VCAN* was associated with high-quality embryo [OR = 1.01 (1.00, 1.02)]	Cohort study Good*
Tanriverdi et al. [40] ●■▲	Women undergoing IVF	_	47 women undergoing IVF	Immunohistochemistry on CC samples	GnRH agonist and gonadotropins	No correlations were observed between notch1, notch2, notch3, notch4, jagged1, and jagged2 expression; mature oocyte number; fertilization rates, and embryo quality percentage	Prospective cohort study Fair*
Taugourdeau et al. [41] ▲	Women undergoing IVF with ICSI	_	71 patients undergoing IVF with ICSI	RT-PCR on 84 CC samples	Recombinant FSH with GnRH antagonist	Increased mtDNA in CGCs was associated with implanted embryos ($p < 10^4$)	Cohort study Fair*

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Tiegs et al. [42] ■	Women undergoing IVF with ICSI, aged between 18 and 44 years, FSH ≤ 12 IU/ml and AFC ≥ 8	BMI ≥ 35 kg/m^2, single gene disorder, or structural chromosomal rearrangement requiring preimplantation genetic testing for monogenetic disorders or preimplantation genetic testing for structural chromosome rearrangements	_	RNA sequencing and RT-PCR on 24 CC samples Individual CCs Endogenous control for *HPRT* and *ACTB*	_	Increased expression of ***CYR61/CCN1* (*p* = 0.04), *DVL2*** (p = 0.04), and ***SRF* (*p* = 0.01)** was associated with aneuploid embryos	Prospective cohort study Fair*
Wathlet et al. [43] ●	Women undergoing IVF with ICSI	-	45 women undergoing IVF with ICSI	RT-PCR on CC samples Individual CCs Endogenous control for *B2M* and *UBC*	GnRH antagonist or agonist	Increased expression of *PTGS2* in mature oocytes Decreased *VCAN* expression in mature oocytes	Cohort study Fair*
Wathlet et al. [44] ▲	Women undergoing IVF with ICSI	-	47 women undergoing IVF with ICSI	RT-PCR on CC samples Individual CCs Endogenous control for *B2M* and *UBC*	GnRH agonist or antagonist	*CAMK1D, EFNB2,* and *STC1* have the highest predictive value for pregnancy outcomes (p = 0.0068)	Cohort study Fair*
Yao et al. [45] ●▲	Women undergoing IVF. Basal follicle-stimulating hormone (day 3 FSH) ≤ 10 IU/ml, first stimulation cycle for IVF-ET, and presence of bilateral ovaries	Ovulatory dysfunction, endometriosis, endocrine disorders such as polycystic ovarian syndrome or hyperprolactinemia, primary infertility, immune infertility, and women taking special drugs or undergoing ovarian surgery	52 women undergoing IVF	RT-PCR on 187 CC samples Individual CCs Endogenous control for *B-Actin*	GnRH agonist long protocol	Decreased mRNA expression of *PTEN* was significantly associated with better oocyte quality (p < 0.001) Increased *PTEN* expression was associated with abnormal fertilization and low-quality blastocyst (p < 0.001)	Prospective cohort study Fair*
Zhang et al. (2005) [46] ▲	Women undergoing IVF	_	_	Microarray and RT-PCR on CC samples Individual CCs Endogenous control for *18S rRNA*	_	Increased expression of PTX3 in CCs from fertilized oocytes (3- to 12-fold increase) (p < 0.01)	Laboratory study

Table 1 (continued)

Author	Inclusion criteria	Exclusion criteria	Population	Method	Protocol	Results	Study type and quality
Zhou et al. [47] ●	Women undergoing IVF with ICSI, 24 to 38 years of age, BMI 17.9–24.9	Endometriosis, polycystic ovary syndrome, uterine fibroids, or ovarian cysts	29 women undergoing IVF with ICSI	RT-PCR on 340 CC samples Endogenous control for *GAPDH*	–	Decreased mRNA expression of ARRB*1* (*P=0.016*), *LGR4* (*P=0.025*), and *SMC2* (P=0.013) was associated with blastocyst development No significant differences in the mRNA levels of *ATP2C1*, *CNTNAP1*, *CDH5*, *MKLN1*, *RHOBTB1*, and *SIX2* in the blastocyst vs non-blastocyst groups	Cohort study Fair*

CCs cumulus cells, *NGS* next-generation sequencing, *RT-PCR* reverse transcription polymerase chain reaction

Shaded circle (●) denotes oocyte quality, shaded square (■) denotes embryo quality, and shaded triangle (▲) denotes IVF clinical outcomes

and embryo quality [24, 39]. Hammond et al. found that increased expression of *VCAN* was significantly associated with good-quality embryos [24], however, Shen et al. showed that decreased expression of *VCAN* was associated with good-quality embryos [39]. Contradictory results could stem from different methods used to isolate cumulus cells (enzymatic denudation using hyaluronidase enzyme vs mechanical denudation, respectively).

Several studies evaluated the predictive value of different biomarkers for embryo quality. Desquiret-Dumas et al. found that mitochondrial DNA (mtDNA) of CCs was a positive predictor of embryo quality (AUC 0.806, 95% CI 0.719–0.869) [18]. Anderson et al. showed a significant negative predictive value of *BDNF* for embryo quality (AUC 0.40, 95% CI 0.32–0.49) [7]. Devjak et al. showed that *AMHR2* could predict embryo quality, the binary logistic regression model yielded AUC 0.69 ± 0.08 [19]. McKenzie et al. also found that increased *HAS2* expression could act as a predictor of embryo quality, AUC 0.76 (95% CI 0.65–0.88) [33]. Baratas et al. also showed that the cumulus cell DNA fragmentation index (CCDFI) was negatively correlated to embryo development [10]. Receiver operator characteristics curves (ROC) suggested that a cut-off value of 20.3% CCDFI with a sensitivity of 71.2% and a specificity of 61% was able to predict embryo quality [10].

Genetic and morphological biomarkers from cumulus cells and clinical outcomes

Several studies examined the role of genetic biomarkers in CCs in assessing clinical outcomes including implantation, pregnancy, fertilization, and live birth rates. Table 4 summarizes the association between CCs biomarkers and IVF clinical outcomes. Twenty-two studies evaluated the association between genetic biomarkers in CCs and IVF clinical outcomes. Six out of 41 biomarkers were examined by two or more studies. These six biomarkers include gene encoding for calcium-binding protein (*CALM1*), for extracellular matrix (*VCAN*), for prostaglandin synthesis (*PTGS2*), for Ephrin B2 receptor (*EFNB2*), for regulation of inflammation (*PTX3*) and for G-protein signaling (*RGS2*). Two studies looked at the association between *CALM1* and pregnancy rate [8, 37]. Although Assidi et al. found a significant association between pregnancy rate and increased *CALM1* expression ($p \leq 0.05$), Papler et al. did not find any association between the two ($p = 0.63$) [8, 37]. The discrepancy in results could stem from different housekeeping genes used as RT-PCR control (ACTB, PPIA vs GAPDH, respectively) [8, 37]. Four studies examined the association between *VCAN* and clinical outcomes [20, 23, 37, 39]. Shen et al. showed that decreased *VCAN* expression in CCs was significantly associated with increased implantation rate ($p < 0.05$) [39]. Both Gebhardt et al. and Ekart et al. showed that increased

VCAN expression was associated with increased pregnancy and live birth rate; ($p = 0.02$) and ($p < 0.05$), respectively [20, 23]. However, Papler et al. did not find any association between *VCAN* and clinical outcomes [37]. The aforementioned studies evaluated individual CC masses, and the difference in gene expression could be due to other factors. For instance, Papler et al. used a needle and glass denudation pipette to separate the cumulus-oocyte complexes, while Shen et al., Gebhardt et al., and Ekart et al. used hyaluronidase enzyme, which could alter gene expression [20, 23, 37]. Another reason for the discrepancy in results could be attributed to the different housekeeping genes used as controls for RT-PCR (*B-Actin* vs *GAPDH* vs *RPL19*, respectively). Two studies evaluated the association between *RGS2* and pregnancy rate [22, 36]. While Feuerstein et al. found a significant positive association between *RGS2* and pregnancy rate ($p < 0.05$) [22], Papler et al. did not [37]. Both studies used different controls for RT-PCR (GAPDH vs NTC, respectively) [22, 37]. Two studies investigated the association between *PTGS2* and clinical outcomes [23, 37]. Gebhardt et al. found a significant positive association between *PTGS2* and pregnancy and live birth rates ($p < 0.02$), but Papler et al. did not find any significant association between the two [23, 37]. The difference in gene expression could be due to the different methods used to separate the cumulus-oocyte complexes. Gebhardt et al. used hyaluronidase enzyme while Papler et al. used the mechanical denudation method [23, 37]. To note, both studies evaluated individual CC masses. Two studies examined the association between *EFNB2* and pregnancy rates [36, 44]. Wathlet et al. found that increased expression of *EFNB2* was significantly associated with pregnancy rate [44], however, Papler et al. showed that decreased expression of *EFNB2* was associated with increased pregnancy rate [36]. Contradictory results could stem from different endogenous genes used as RT-PCR control (*UBC*, *B2M* vs *GAPDH*, respectively) and different methods used to isolate cumulus cells (enzymatic denudation using cumulase enzyme vs mechanical denudation using needle and glass denudation pipette). Two studies evaluated the association between *PTX3* and clinical outcomes [23, 46]. Gebhardt et al. found that 2.1-fold increased expression of *PTX3* in CCs was associated with increased live birth rate, although not significant ($p = 0.06$) [23]. Zhang et al. found that increased expression of *PTX3* was significantly associated with increased fertilization rate ($p < 0.01$) [46].

Several studies evaluated the predictive value of different biomarkers for clinical outcomes. Anderson et al. showed a predictive value of *BDNF* for normal fertilization of AUC 0.39 ($p = 0.001$) [7]. The area under the ROC curve of *GDF9* mRNA for pregnancy prediction was 0.816 (0.757–0.875) with a cut-off value of 4.82, a sensitivity of 82%, and a specificity of 64% [30]. The area under the ROC curve of *BMP15* mRNA for pregnancy prediction was 0.746 (0.671–0.821) with a cut-off value of 2.60, a sensitivity of 78%, and a specificity of 52% [30]. *GDF9* and *BMP15* genes belonging to the TGF- ß family were positively associated with pregnancy rate ($p < 0.05$) [30]. A live birth prediction model established by Wathlet et al. showed that the combination of the following genes (*EFNB2*, *CAMK1D*, *STC1*, *GPX3*, and *GSTA3*) resulted in an AUC of 0.93 [44].

Discussion

This systematic review summarized the role of multiple biomarkers of CCs in assessing IVF outcomes, including oocyte quality, embryo quality, and clinical outcomes such as fertilization, implantation, pregnancy, and live birth rates. Most pertinent findings include the positive correlation between oocyte quality and increased mRNA expression of genes encoding for glucose metabolism (*PFKP*), calcium homeostasis (*CAMK1D*), extracellular matrix (*HAS2*, *VCAN*), TGF-ß family (*BMP15*, *GDF9*), and prostaglandin synthesis *(PTGS2)*. Genes encoding for glucose metabolism are important for oocyte maturation as the metabolic demands of CCs increase to help supply the oocyte maturation process [39]. Genes encoding for calcium homeostasis are crucial for sustaining different metabolic functions leading to competent oocytes. For instance, *CAMK1D*, which is a gene encoding for a member of the Ca/Camodulin-dependent protein kinase 1 subfamily of serine/threonine kinases, has been involved in many regulatory processes in the CCs such as regulation of steroidogenesis during folliculogenesis as well as glucose metabolism [38]. It has also been suggested that *CAMK1D* has anti-apoptotic properties which might also assist the oocyte maturation process [38]. The upregulation of both the *PTGS2* gene encoding for prostaglandin synthesis and of *HAS2* as well as *VCAN*—genes encoding for extracellular matrix—contributes to CCs expansion during meiotic resumption and oocyte maturation [38]. BMP-15 protein binds to the receptors bone morphogenetic protein receptor type II (BMPRII) and ALK6, whereas GDF-9 binds to TGF-ß type I receptor kinase (ALK5) and BMPRII. Increased levels of both BMP-15 and GDF-9 result in the development of oocyte and embryonic competence by activating the SMAD signaling pathway [3]. The SMAD pathway induces the expression of several transcripts in the cumulus cells involved in extracellular matrix synthesis (*HAS2*) and steroidogenesis which might promote good-quality embryo development [3]. Out of the 30 biomarkers studied for the assessment of oocyte quality, only 3 genetic biomarkers have been addressed in two or more studies. The lack of reproducibility of results as well as the differences in study methods make it difficult to validate the

Table 2 Summary of studies that evaluated the association of genetic biomarkers from cumulus cells with oocyte quality

Authors	CCs sample size	Oocyte quality	Biomarkers	Mature/ competent oocytes	Immature/ incompetent oocytes
DNA biomarkers					
Cheng et al. [13]	350*	Mature oocyte defined by the presence of the first polar body	Telomere length	↑	↓
RNA biomarkers					
Anderson et al. [7]	674*	Mature oocyte defined by the presence of the first polar body and the absence of germinal vesicle	*PTGS2* mRNA	↑	↓
Bartolucci et al. [11]	-	*Mature oocytes in metaphase II*	*MIR-21-5p*	↑	↓
Daei-Farshbaf et al. [15]	-	Mature oocyte defined by the presence of the first polar body	*PPP3CB mRNA*	↑	↓
Ekart et al. [20]	270*	*Mature oocytes in metaphase II*	*VCAN* mRNA	↓	↑
			HAS2 mRNA	↓	↑
Ito et al. [26]	43	-	*GSTT1* mRNA	↓	↑
Li et al. [30]	242	Mature oocyte defined by the presence of the first polar body	*GDF9* mRNA	↑	↓
			BMP15 mRNA	↑	↓
Li et al. [31]	308	Mature oocyte defined by the presence of the first polar body	*GJA1* mRNA	↓	↑
			SERPINE2 mRNA	↓	↑
Montazeri et al. [34]	-	-	*AMHR2* mRNA	↓	↑
			FSHR mRNA	↓	↑
Scarica et al. [38]	75*	-	*PTGS2* mRNA	↑	↓
			CAMK1D mRNA	↑	↓
			HAS2 mRNA	↑	↓
Shen et al. [39]	354*	Mature oocyte defined by the presence of the first polar body	*PFKP* mRNA	↑	↓
			PKM2 mRNA	↑	↓
			VCAN mRNA	↓	↑
Wathlet et al. [43]	42*	Fertilization or 2 PN	*PTGS2* mRNA	↑	↓
			VCAN mRNA	↓	↑
Yao et al. [45]	187*	-	*PTEN* mRNA	↓	↑
Zhou et al. [47]	340	-	*ARRB1 mRNA*	↓	↑
			LGR4 mRNA	↓	↑
			SMC2 mRNA	↓	↑
Protein biomarkers					
Alfaidy et al. [6]	52*	Oocyte competence defined as the ability to reach the blastocyst stage	PROK1	↑	↓
Demiray et al. [17]	60*	Mature oocyte defined by the presence of the first polar body	BMP2	↑	↓
Tanriverdi et al. [40]	-	Mature MII oocytes defined as having clear cytoplasm, normal cell size, normal zona pellucida, and non-fragmented polar body	Notch1, notch2, notch3, notch4, jagged1, and jagged2	-	-
Degradation biomarkers					
Baratas et al. [10]	130*	-	CCDFI	↓	↑
Bosco et al. [12]	-	-	pAKT/DFI ratio	↑	↓

CCDFI cumulus cells DNA fragmentation index, *CC* cumulus cells, *ICSI* intracytoplasmic sperm injection, *IVF* in-vitro fertilization, *mtDNA* mitochondrial DNA, *MII* metaphase II, *PGT-A* preimplantation genetic testing for aneuploidy, *RT-PCR* reverse transcription polymerase chain reaction

Bold arrows denote statistically significant results

*Individual CCs

Table 3 Summary of studies that evaluated the association of genetic biomarkers from cumulus cells with embryo quality

Authors	CCs sample size	Embryo quality	Biomarkers	Good-quality/ euploid embryos	Poor-quality/ aneuploid embryos
DNA biomarkers					
Cheng et al. [13]	350*	Good-quality embryos defined as grade 1 and 2, with at least eight blastomeres and < 20% cytoplasmic fragmentation. Poor-quality embryos defined as grade 3 and 4, with an unequal blastomere size and 20–50% fragmentation	Telomere length	↑	↓
Desquiret-Dumas [18]	452*	Embryos scored according to ESHRE consensus depending on the cell number, the stage-specific cell size, the percentage of fragmentation, and the existence of multinucleation	mtDNA	↑	↓
RNA biomarkers					
Anderson et al. [7]	674*	Grade 1 symmetrical blastomeres and absent fragmentation, grade 2 slightly uneven blastomeres and < 20% fragmentation, grade 3 uneven blastomeres and > 20% fragmentation	*GREM1* mRNA	↑	↓
			PTGS2 mRNA	↑	↓
			BDNF mRNA	↓	↑
Cillo et al. [14]	90*	Grading system from 1 to 5 depending on equal-sized blastomeres, pattern of fragmentation, and cytoplasmic appearance	*HAS2* mRNA	↑	↓
			GREM1 mRNA	↑	↓
Devjak et al. [19]	55*	-	*AMHR2* mRNA	↓	↑
Hammond et al. [24]	24*	Blastocysts were graded on day 5 according to the degree of expansion of the blastocoel cavity and the number and quality of inner cell mass and trophectoderm	*ATP51* mRNA	↑	↓
			HSPA5, PRDX3, VCAN mRNA	↓	↑
Hasegawa et al. [25]	135*	Embryo quality was categorized as good morphology if the number of blastomeres was greater than seven cells with less than 5% fragmentation	*PR* mRNA	↓	↑
Kordus et al. [28]	163*	Embryo euploidy determined by PGT-A	*HSD3B1* mRNA	↓	↑
Lee et al. [29]	24	On day 3, grades 1 and 2 were good-quality embryos with at least eight blastomeres and less than 20% cytoplasmic fragmentation; grades 3 and 4 were poor-quality embryos with unequal blastomere size and 20-50% fragmentation	*CKB* mRNA	↑	↓
			PRDX2 mRNA	↑	↓
Li et al. [30]	2426	A high-quality embryo should consist of 7–9 blastomeres with a uniform size, and the fragment proportion should be less than 10%	*GDF9* mRNA	↑	↓
			BMP15 mRNA	↑	↓

Table 3 (continued)

Authors	CCs sample size	Embryo quality	Biomarkers	Good-quality/ euploid embryos	Poor-quality/ aneuploid embryos
Li et al. [31]	308*	Good quality (grade 1 and 2) grade 1, even-sized blastomeres with no cytoplasmic fragmentation; grade 2, even-sized blastomeres with minor cytoplasmic fragmentation ≤ 20 % of the embryo surface; poor-quality (grade 3 to 5) grade 3, uneven-sized blastomeres with variable fragmentation; grade 4, even—or uneven-sized blastomeres with moderate to significant cytoplasmic fragmentation > 20 % of the embryo surface; and grade 5, few blastomeres of any size and severe fragmentation ≥ 50 % of the embryo surface	*GJA1, SERPINE2, PRSS35,* and *PTX3* mRNA	-	-
McKenzie et al. [33]	108	Embryos were graded according to a standard institutional grading scale	*HAS2, PTGS2, GREM1* mRNA	↑	↓
Shen et al. [39]	354*	High-quality (grades 1 and 2) defined as more than six blastomeres of regular size and less than 25% fragmentation	*VCAN* mRNA	↑	↓
Tiegs et al. [42]	24*	Embryo euploidy determined by PGT-A	*CYR61/CCN1 mRNA*	↓	↑
			DVL2 mRNA	↓	↑
			SRF mRNA	↓	↑
Yao et al. [45]	187*	High-quality embryo on day 3 defined as 6–10 blastomeres with uniform sizes and the fragment proportion should be less than 20%	*PTEN* mRNA	↓	↑
Protein biomarkers					
Demiray et al. [17]	60*	Good quality embryos included grades 1 and ½ embryos, medium quality embryos included grades 2 and 2/3, poor quality embryos included grades 3 and 4	BMP2	↑	↓
Fang et al. [21]	-	Grade 1–2, equally sized blastomeres and 0–20% fragmentation; grade 3–4, blastomeres of unequal sizes and 20% fragmentation on day 3	Cited2	↓	↑
Hasegawa et al. [25]	135*	Embryo quality was categorized as good morphology if the number of blastomeres was greater than seven cells with less than 5% fragmentation	PR	↓	↑
Tanriverdi et al. [40]	-	Grade 1 embryos with equally sized blastomeres and without cytoplasmic fragmentation, grade 2 embryos with equally sized blastomeres and minor cytoplasmic fragmentations, grade 3 embryos without equally sized blastomeres and without cytoplasmic fragmentations, grade 4 embryos with or without equally sized blastomeres and with major cytoplasmic fragmentations, grade 5 embryos with blastomeres that cannot be distinguished and with major cytoplasmic fragmentations	Notch1, notch2, notch3, notch4, jagged1, and jagged2	-	-

Table 3 (continued)

Authors	CCs sample size	Embryo quality	Biomarkers	Good-quality/ euploid embryos	Poor-quality/ aneuploid embryos
Degradation biomarkers					
Baratas et al. [10]	130*	-	CCDFI	↑	↑

CCDFI cumulus cells DNA fragmentation index, *CC* cumulus cells, *CGC* cumulus granulosa cells, *ICSI* intracytoplasmic sperm injection, *IVF* in-vitro fertilization, *mtDNA* mitochondrial DNA, *PGT-A* preimplantation genetic testing for aneuploidy, *RT-PCR* reverse transcription polymerase chain reaction

Bold arrows indicate statistically significant results

*Individual CCs

association between the studied biomarkers and oocyte quality.

A positive correlation was found between embryo quality and increased mRNA expression of genes encoding for extracellular matrix (*VCAN*, *HAS2*), hormone receptor (*AMHR2*), prostaglandin synthesis (*PTGS2*), and *GREM1*. *PTGS2* is a gene encoding for an enzyme involved in prostaglandin synthesis and is important for oocyte maturation and eventually good-quality embryo formation [7]. *GREM1* is a BMP antagonist and plays a role in embryonic development. Curran et al. showed that mice embryonic fibroblasts (MEFs) lacking both copies of *GREM1* resulted in increased cell growth and proliferation in the absence or presence of growth factors and accelerated wound closure. It is also involved in the limb development process and angiogenic sprouting of endothelial cells [48]. Low expression of genes encoding for hormone receptors such as *AMHR2* is indicative of good oocyte quality because AMH triggers primordial follicle recruitment [34]. This function is not needed once maturity is reached, hence the low expression of *AMHR2* in CCs. This also shows that oocyte maturity is a prerequisite for high-quality embryo formation. Only two biomarkers for assessment of embryo quality have been studied in two or more studies and they both highlight the positive association between *GREM1*, *PTGS2*, and embryo quality. However, more studies are needed to replicate the results and find additional biomarkers that could accurately assess embryo quality.

A positive correlation was also found between pregnancy rate and genes encoding for calcium-binding protein (*CALM1*), extracellular matrix (*VCAN*), regulation of G-protein signaling (*RGS2*), prostaglandin synthesis (*PTGS2*), Ephrin B2 receptor (*EFNB2*), and regulation of inflammation (*PTX3*). *CALM1* is a gene that encodes for calcium-binding protein and it is associated with increased pregnancy rate, however, its predictive value for pregnancy outcomes remains debatable. Downstream targets of *GDF-9* including *PTGS2* were correlated with increased pregnancy rate which aligns with the findings of a systematic review conducted by Sirait et al. found that increased expression of genes belonging to the TGF- ß family *(GDF9, BMP15)* in cumulus cells was associated with good-quality oocyte and increased pregnancy rate [4]. Increased expression of the TGF- ß family (*GDF9, BMP15*) results in the activation of the SMAD signaling pathway which promotes the expression of several genes encoding for steroidogenesis and extracellular matrix synthesis which are associated with good-quality embryo and increased pregnancy rate. Interestingly, Ocampo et al. proposed a model to assess the association between the combination of *PTGS2* and *VCAN* with pregnancy rate and showed that a high PVL index—which evaluates increased expression of both genes—was associated with increased clinical pregnancy [49]. Additional studies are needed to

Table 4 Summary of studies that evaluated the association of genetic biomarkers from cumulus cells with IVF clinical outcomes

Authors	CCs sample size	Biomarkers	Fertilization rate	Implantation rate	Pregnancy rate	Live birth rate
DNA biomarkers						
Cheng et al. [13]	350*	Telomere length	↑	-		
Taugourdeau et al. [41]	84	mtDNA	-	↑	-	-
RNA biomarkers						
Anderson et al. [7]	674 *	*BDNF* mRNA	↓	-	-	-
		GREM 1 mRNA	-	-	↑	-
Assidi et al. [8]	6 *	*DPP8*, *HIST1H4C*, *UBQLN1*, *CALM1*, *NRP1*, *PSMD6* mRNA	-	-	↑	-
Assou et al. [9]	50*	*BCL2L11*, *PCK1* mRNA	-	-	↑	-
		NFIB mRNA	-	-	↓	-
Daei-Farshbaf et al. [15]	-	*OR10H2* mRNA	↑	-	-	-
Ekart et al. [20]	270*	*VCAN mRNA*	-		-	↑
Feuerstein et al. [22]	56*	*RGS2 mRNA*	-	-	↑	-
Gebhardt et al. [23]	38*	*PTGS2*, *VCAN mRNA*	-	-	↑	↑
		PTX3 mRNA	-	-	-	↑
Kim et al. [27]	-	*LDLR, StAR* mRNA	-	-	↑	-
		ADCY and *HSD17B* mRNA		-	↓	-
Kordus et al. [28]	163	*PAPPA* mRNA	-	-	-	↑
		AREG mRNA	-	-	-	↓
Li et al. [30]	2426	*GDF9* mRNA	↑	-	↑	-
		BMP15 mRNA	↑	-	↑	-
Li et al. [31]	308*	*PRSS35* mRNA	↑	-	-	-
Papamentzelopoulou et al. [35]	-	*LHR* mRNA	-	-	↑	-
Papler et al. [36]	43*	*EFNB2* mRNA	-	-	↓	-
		RGS2 and *VCAN* mRNA	-	-	-	-
Papler et al. [37]	55*	*CALM1*, *HIPK1*, *ITM2A*, *KHDRBS3*, *KRT6A*, *NUDT10*, *PTGS2*, *TBX6*, *TMEM64, WRB* mRNA	-	-	-	-
Shen et al. [39]	354*	*VCAN*, *PKM2* mRNA		↓		
Wathlet et al. [44]	47*	*EFNB2*, *CAMK1D*, *GSTA4*, *GSR* mRNA		-	↑	
Yao et al. [45]	187*	*PTEN* mRNA	↓	-	-	-
Zhang et al. [46]	98*	*PTX3* mRNA	↑	-	-	-
Protein biomarkers						
Fang et al. [21]	-	Cited2	↓	↓	↓	-
Matos et al. [32]	-	SOD	-	-	↑	-

CC cumulus cells, *ICSI* intracytoplasmic sperm injection, *IVF* in-vitro fertilization, *PGT-A* preimplantation genetic testing for aneuploidy, *RT-PCR* reverse transcription polymerase chain reaction, *NGS* next-generation sequencing

Bold arrows indicate statistically significant results

*Individual CCs

validate the predictive value of the biomarkers associated with clinical outcomes.

A number of studies quantitated the association between genetic biomarkers in CCs and oocyte quality, embryo quality, and IVF clinical outcomes. However, only 10 out of 42 studies validated this association and predictive value using statistical tests such as correlation coefficient and AUC.

Studies included in this review evaluated the association between a variety of genetic biomarkers and oocyte quality, embryo quality, and IVF clinical outcomes including fertilization, implantation, pregnancy, and live birth rates. Despite the evident strengths of this review like the comprehensiveness, there are certain limitations. The outcomes of interests including oocyte quality, embryo quality, and clinical outcomes are

defined with variability among different studies which limits cross-study comparison and meta-analysis performance. The inclusion of low-quality studies is another potential limitation of this study. Although all studies reported the IVF protocols utilized, the use of different protocols makes it difficult to evaluate their effect on IVF outcomes. The sample size used in 10 out of 42 studies is small, which could hinder the generalizability of the results. Also, patient characteristics differ among studies which adds to the heterogeneity of the presented data. Finally, CCs were obtained variably in the included papers. Some CCs were exterior cells cut away from the oocyte with needles, others were removed by treatment with hyaluronidase. The latter group contains CCs with intimate contact with the oocytes whereas the others do not.

Conclusion and future perspective

The findings detailed in this systematic review highlight an important correlation between biomarkers from cumulus cells and oocyte/embryo quality, as well as IVF clinical outcomes. These findings could help to develop a larger prospective study that would help determine the predictive value of CC biomarker levels whether individually or combined to potentially devise tests for cumulus cell biomarkers that would help select the best quality oocyte or embryo for uterine transfer with the highest odds of yielding pregnancy and live birth. Analyzing CC biomarkers offers a non-invasive approach to understanding oocyte and embryo quality and implementing this method would help fulfill the growing need to identify the best quality oocyte for cryopreservation and donor egg banking as well as the best quality embryo for freezing and successful clinical outcomes. Biomarkers evaluated in this review could potentially complement current morphological and genetic criteria to determine the quality of oocytes, embryos, and IVF outcomes.

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Data availability The data that support the findings of this study were identified from the following resources available in the public domain: PubMed, Embase, Scopus, Cochrane Library, and Web of Science.

Code availability N/A

Declarations

Competing interests The authors declare no competing interests.

References

1. Ozturk S. Selection of competent oocytes by morphological criteria for assisted reproductive technologies. Mol Reprod Dev. 2020;87(10):1021–36. https://doi.org/10.1002/mrd.23420.
2. Gardner DK, Balaban B. Assessment of human embryo development using morphological criteria in an era of time-lapse, algorithms and 'OMICS': is looking good still important? Mol Hum Reprod. 2016;22(10):704–18. https://doi.org/10.1093/molehr/gaw057.
3. Turathum B, Gao EM, Chian RC. The function of cumulus cells in oocyte growth and maturation and in subsequent ovulation and fertilization. Cells. 2021;10(9) https://doi.org/10.3390/cells10092292.
4. Sirait B, et al. Oocyte competence biomarkers associated with oocyte maturation: a review. Front Cell Dev Biol. 2021;9:710292. https://doi.org/10.3389/fcell.2021.710292.
5. Moher D, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 2009;339:b2535. https://doi.org/10.1136/bmj.b2535.
6. Alfaidy N, et al. Prokineticin 1 is a new biomarker of human oocyte competence: expression and hormonal regulation throughout late folliculogenesis. Biol Reprod. 2019;101(4):832–41. https://doi.org/10.1093/biolre/ioz114.
7. Anderson RA, et al. Cumulus gene expression as a predictor of human oocyte fertilisation, embryo development and competence to establish a pregnancy. Reproduction. 2009;138(4):629–37. https://doi.org/10.1530/REP-09-0144.
8. Assidi M, et al. Biomarkers of human oocyte developmental competence expressed in cumulus cells before ICSI: a preliminary study. J Assist Reprod Genet. 2011;28(2):173–88. https://doi.org/10.1007/s10815-010-9491-7.
9. Assou S, et al. A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. Mol Hum Reprod. 2008;14(12):711–9. https://doi.org/10.1093/molehr/gan067.
10. Baratas A, et al. Cumulus cell DNA damage as an index of human oocyte competence. Reprod Sci. 2022;29(11):3194–200. https://doi.org/10.1007/s43032-021-00817-7.
11. Bartolucci AF, Uliasz T, Peluso JJ. MicroRNA-21 as a regulator of human cumulus cell viability and its potential influence on the developmental potential of the oocyte. Biol Reprod. 2020;103(1):94–103. https://doi.org/10.1093/biolre/ioaa058.
12. Bosco L, et al. Relationship between apoptosis and survival molecules in human cumulus cells as markers of oocyte competence. Zygote. 2017;25(5):583–91. https://doi.org/10.1017/S0967199417000429.
13. Cheng EH, et al. Evaluation of telomere length in cumulus cells as a potential biomarker of oocyte and embryo quality. Hum Reprod. 2013;28(4):929–36. https://doi.org/10.1093/humrep/det004.
14. Cillo F, et al. Association between human oocyte developmental competence and expression levels of some cumulus genes. Reproduction. 2007;134(5):645–50. https://doi.org/10.1530/REP-07-0182.
15. Daei-Farshbaf N, et al. Expression pattern of olfactory receptor genes in human cumulus cells as an indicator for competent oocyte selection. Turk J Biol. 2020;44(6):371–80. https://doi.org/10.3906/biy-2003-79.
16. Daei-Farshbaf N, et al. Identification of calcineurin as a predictor of oocyte quality and fertilization competence based on microarray data. Comput Biol Chem. 2021;94:107561. https://doi.org/10.1016/j.compbiolchem.2021.107561.
17. Demiray SB, et al. Expression of the bone morphogenetic protein-2 (BMP2) in the human cumulus cells as a biomarker of oocytes and embryo quality. J Hum Reprod Sci. 2017;10(3):194–200. https://doi.org/10.4103/jhrs.JHRS_21_17.
18. Desquiret-Dumas V, et al. The mitochondrial DNA content of cumulus granulosa cells is linked to embryo quality. Hum Reprod. 2017;32(3):607–14. https://doi.org/10.1093/humrep/dew341.

19. Devjak R, et al. Embryo quality predictive models based on cumulus cells gene expression. Balkan J Med Genet. 2016;19(1):5–12. https://doi.org/10.1515/bjmg-2016-0001.
20. Ekart J, et al. Ranking and selection of MII oocytes in human ICSI cycles using gene expression levels from associated cumulus cells. Hum Reprod. 2013;28(11):2930–42. https://doi.org/10.1093/humrep/det357.
21. Fang Y, et al. Cited2 protein level in cumulus cells is a biomarker for human embryo quality and pregnancy outcome in one in vitro fertilization cycle. Fertil Steril. 2016;105(5):1351-1359 e4. https://doi.org/10.1016/j.fertnstert.2015.12.137.
22. Feuerstein P, et al. Genomic assessment of human cumulus cell marker genes as predictors of oocyte developmental competence: impact of various experimental factors. PLoS One. 2012;7(7):e40449. https://doi.org/10.1371/journal.pone.0040449.
23. Gebhardt KM, et al. Human cumulus cell gene expression as a biomarker of pregnancy outcome after single embryo transfer. Fertil Steril. 2011;96(1):47-52 e2. https://doi.org/10.1016/j.fertnstert.2011.04.033.
24. Hammond ER, et al. Assessing embryo quality by combining non-invasive markers: early time-lapse parameters reflect gene expression in associated cumulus cells. Hum Reprod. 2015;30(8):1850–60. https://doi.org/10.1093/humrep/dev121.
25. Hasegawa J, et al. Reduction of progesterone receptor expression in human cumulus cells at the time of oocyte collection during IVF is associated with good embryo quality. Hum Reprod. 2005;20(8):2194–200. https://doi.org/10.1093/humrep/dei005.
26. Ito M, et al. Glutathione S-transferase theta 1 expressed in granulosa cells as a biomarker for oocyte quality in age-related infertility. Fertil Steril. 2008;90(4):1026–35. https://doi.org/10.1016/j.fertnstert.2007.07.1389.
27. Kim MJ, et al. Upregulation of low-density lipoprotein receptor of the steroidogenesis pathway in the cumulus cells is associated with the maturation of oocytes and achievement of pregnancy. Cells. 2021;10(9) https://doi.org/10.3390/cells10092389.
28. Kordus RJ, et al. Cumulus cell pappalysin-1, luteinizing hormone/choriogonadotropin receptor, amphiregulin and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 mRNA levels associate with oocyte developmental competence and embryo outcomes. J Assist Reprod Genet. 2019;36(7):1457–69. https://doi.org/10.1007/s10815-019-01489-8.
29. Lee MS, et al. Association of creatin kinase B and peroxiredoxin 2 expression with age and embryo quality in cumulus cells. J Assist Reprod Genet. 2010;27(11):629–39. https://doi.org/10.1007/s10815-010-9459-7.
30. Li Y, et al. Increased GDF9 and BMP15 mRNA levels in cumulus granulosa cells correlate with oocyte maturation, fertilization, and embryo quality in humans. Reprod Biol Endocrinol. 2014;12:81. https://doi.org/10.1186/1477-7827-12-81.
31. Li SH, et al. Correlation of cumulus gene expression of GJA1, PRSS35, PTX3, and SERPINE2 with oocyte maturation, fertilization, and embryo development. Reprod Biol Endocrinol. 2015;13:93. https://doi.org/10.1186/s12958-015-0091-3.
32. Matos L, et al. Superoxide dismutase expression in human cumulus oophorus cells. Mol Hum Reprod. 2009;15(7):411–9. https://doi.org/10.1093/molehr/gap034.
33. McKenzie LJ, et al. Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum Reprod. 2004;19(12):2869–74. https://doi.org/10.1093/humrep/deh535.
34. Montazeri F, et al. Association between cumulus cells—mRNA levels of AMHR2 and FSHR with oocyte maturity. Middle East Fertil Soc J. 2022;27(1):26. https://doi.org/10.1186/s43043-022-00116-4.
35. Papamentzelopoulou M, et al. LH receptor gene expression in cumulus cells in women entering an ART program. J Assist Reprod Genet. 2012;29(5):409–16. https://doi.org/10.1007/s10815-012-9729-7.
36. Burnik Papler T, et al. Specific gene expression differences in cumulus cells as potential biomarkers of pregnancy. Reprod BioMed Online. 2015;30(4):426–33. https://doi.org/10.1016/j.rbmo.2014.12.011.
37. Burnik Papler T, et al. No specific gene expression signature in human granulosa and cumulus cells for prediction of oocyte fertilisation and embryo implantation. PLoS One. 2015;10(3):e0115865. https://doi.org/10.1371/journal.pone.0115865.
38. Scarica C, et al. An integrated investigation of oocyte developmental competence: expression of key genes in human cumulus cells, morphokinetics of early divisions, blastulation, and euploidy. J Assist Reprod Genet. 2019;36(5):875–87. https://doi.org/10.1007/s10815-019-01410-3.
39. Shen Q, et al. Versican expression level in cumulus cells is associated with human oocyte developmental competence. Syst Biol Reprod Med. 2020;66(3):176–84. https://doi.org/10.1080/19396368.2020.1725685.
40. Tanriverdi G, et al. Notch signaling pathway in cumulus cells can be a novel marker to identify poor and normal responder IVF patients. J Assist Reprod Genet. 2013;30(10):1319–26. https://doi.org/10.1007/s10815-013-0072-4.
41. Taugourdeau A, et al. The mitochondrial DNA content of cumulus cells may help predict embryo implantation. J Assist Reprod Genet. 2019;36(2):223–8. https://doi.org/10.1007/s10815-018-1348-5.
42. Tiegs AW, et al. Cumulus cells of euploid versus whole chromosome 21 aneuploid embryos reveal differentially expressed genes. Reprod BioMed Online. 2021;43(4):614–26. https://doi.org/10.1016/j.rbmo.2021.06.015.
43. Wathlet S, et al. Cumulus cell gene expression predicts better cleavage-stage embryo or blastocyst development and pregnancy for ICSI patients. Hum Reprod. 2011;26(5):1035–51. https://doi.org/10.1093/humrep/der036.
44. Wathlet S, et al. Pregnancy prediction in single embryo transfer cycles after ICSI using QPCR: validation in oocytes from the same cohort. PLoS One. 2013;8(4):e54226. https://doi.org/10.1371/journal.pone.0054226.
45. Yao J, et al. PTEN expression in human cumulus cells is associated with embryo development competence. Zygote. 2022;30(5):611–8. https://doi.org/10.1017/S096719942200003X.
46. Zhang X, et al. Studies of gene expression in human cumulus cells indicate pentraxin 3 as a possible marker for oocyte quality. Fertil Steril. 2005;83(Suppl 1):1169–79. https://doi.org/10.1016/j.fertnstert.2004.11.030.
47. Zhou T-P, et al. Expression of target genes in cumulus cells derived from human oocytes with and without blastocyst formation. Reprod Dev Med. 2019;3(2):84–8. https://doi.org/10.4103/2096-2924.262387.
48. Curran SP, et al. Deletion of Gremlin1 increases cell proliferation and migration responses in mouse embryonic fibroblasts. Cell Signal. 2012;24(4):889–98. https://doi.org/10.1016/j.cellsig.2011.12.008.
49. Ocampo A, et al. Assessment of prostaglandin-endoperoxide synthase 2 and versican gene expression profile from the cumulus cells: association with better in vitro fertilization outcomes. J Ovarian Res. 2018;11(1):84. https://doi.org/10.1186/s13048-018-0456-2.