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RESEARCH

Addition of GM-CSF during *in vitro* oocyte maturation improves embryo development and implantation and birth rate in mice

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Abstract

The present study determined whether adding granulocyte–macrophage colony-stimulating factor (GM-CSF) during *in vitro* oocyte maturation (IVM) could improve oocyte developmental competence by examining embryo development and implantation and birth rates following embryo transfer in mice. In an initial dose-response experiment, we demonstrated that the addition of 2 and 10 ng/mL GM-CSF during IVM increased cumulus expansion ($P < 0.05$) but did not affect fertilisation rate compared with the control group. The addition of 10 ng/mL increased blastocyst rate (17.0%; $P < 0.05$) and tended to increase the number of good quality blastocysts present at 96 h of culture (+19.4%; $P = 0.06$) and increased blastocyst inner cell mass (+25.2%; $P < 0.001$), trophectoderm (+29.9%; $P < 0.01$), and total cell numbers (+28.6%; $P < 0.05$). GM-CSF also reduced the incidence of DNA damage in blastocysts in the 10 ng/mL group (−16.2%) compared with the control group. These improvements translated into increases in implantation rate (+21.0%; $P < 0.05$) and birth rate (+17.0%; $P < 0.001$) following the transfer of vitrified blastocysts. GM-CSF treatment did not alter any fetal and placental parameters. Together these results suggest that the addition of GM-CSF during IVM may improve livestock *in vitro* embryo production and human IVM.

Lay summary

The ability to collect immature eggs from the ovaries and mature these in the laboratory is an important technology for treating certain types of infertility in women as well as for preserving their fertility, for example prior to cancer treatment. This technique is called *in vitro* oocyte maturation or IVM and is also used in animal breeding. However, pregnancy and birth rates in humans and animals using this technique are lower than that which can be achieved using natural mating. We have shown that adding GM-CSF, a molecule found in the ovary, during IVM can increase the number and quality of embryos produced in mice. We have also found that when these embryos are transferred to surrogate mothers, implantation and birth rates are increased. These results suggest that the addition of GM-CSF during IVM may improve pregnancy and birth rates in humans as well as animals.

Keywords: birth rate; GM-CSF; granulocyte–macrophage colony-stimulating factor; implantation rate; in vitro oocyte maturation; IVM; preimplantation embryo development

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Introduction

In vitro oocyte maturation (IVM) is an integral component of *in vitro* embryo production (IVP) for livestock breeding, in particular cattle (Ferré *et al.* 2020). IVM is also used as an assisted reproductive technology (ART) where controlled ovarian stimulation is contraindicated for patients with a high antral follicle count, especially patients with polycystic ovaries or polycystic ovary syndrome, as well as for fertility preservation, including oncology patients (Gilchrist & Smitz 2023). However, IVM is not as successful as *in vivo* maturation and continues to be an area of ongoing research.

Numerous studies have shown that the addition of granulocyte–macrophage colony-stimulating factor (GM-CSF), a cytokine secreted by the epithelial cells in the female reproductive tract, during *in vitro* embryo culture (IVC) improves embryo development in a range of species including mice (Robertson *et al.* 2001), cattle (de Moraes & Hansen, 1997) and humans (Sjöblom *et al.* 1999). These improvements have also been shown to result in increased implantation rates as well as birth rates of around 20% in mice (Sjoblom *et al.* 2005), cattle (Loureiro *et al.* 2009) and humans (Ziebe *et al.* 2013).

Granulocyte–macrophage colony-stimulating factor is also present in the ovarian follicle (Jasper *et al.* 1996); however, whether it can improve IVM has not been widely studied. In the only full report, Peralta *et al.* (2013) examined the effect of adding 1, 10, and 100 ng/mL recombinant human GM-CSF during bovine IVM and showed that 100 ng/mL increased cumulus cell expansion and proliferation but did not affect nuclear or cytoplasmic maturation, cleavage rate, or blastocyst development. However, the use of recombinant human GM-CSF may have limited its effect, as this only shares 71% homology with bovine GM-CSF, which has been shown to limit its cross-reactivity in bioassays (Maliszewski *et al.* 1988).

The aim of the present study, therefore, was to determine whether the addition of species-specific GM-CSF during IVM could improve oocyte developmental competence by examining preimplantation embryo development in mice. This was done using a range of doses shown previously to improve embryo culture in mice and humans (2 ng/mL; Sjöblom *et al.* 1999, Robertson *et al.* 2001) and cattle (10 ng/mL; de Moraes & Hansen 1997) before examining implantation and birth rates following the transfer of vitrified blastocysts to recipient mice.

Materials and methods

All chemicals were purchased from Sigma-Aldrich unless otherwise stated. Culture media and reagents were prepared in-house or, where specified, obtained from Cook Medical (Bloomington, Indiana, USA).

Mice

CBAF1 males (6–8 weeks) and females (21–23 days) were housed under a 12-h light and 12-h darkness cycle with a regular supply of water and food and allowed to feed *ad libitum*. All experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the study was approved by the University of Adelaide Animal Ethics Committee (M-2017-081).

In vitro oocyte maturation

Female mice were administered 5 IU pregnant mare chorionic gonadotropin (PMSG; Folligon, Intervert, Boxmeer, The Netherlands) via intraperitoneal injection to stimulate follicle growth. All *in vitro* oocyte maturation was performed at 6% CO_2, 5% O_2 and 89% N_2 humidified at 37°C with media pre-equilibrated for at least 4 h prior. 46–48 h post PMSG ovaries were collected in HEPES-buffered minimum essential medium (alpha (α) MEM) handling media (HM; Lim *et al.* 2021) supplemented with 4 mg/mL bovine serum albumin (BSA; MP Biomedicals, AlbumiNZ, Auckland, NZ) and 1 mg/mL fetuin. Cumulus–oocyte complexes (COCs) were isolated from ovaries by puncturing all follicles 100–180 µm in diameter using a 30-gauge needle. Ten COCs were then cultured in 50 µL drops of bicarbonate-buffered α-MEM containing 3 mg/mL BSA and 1 mg/mL Fetuin (Lim *et al.* 2021). The media was filtered before adding 50 mIU/mL recombinant human FSH (Puregon; Organon, Oss, the Netherlands; Lim *et al.* 2021) containing 0, 2, or 10 ng/mL of recombinant mouse GM-CSF (R&D Systems) for 16 h. GM-CSF was reconstituted as per the manufacturer's instructions in PBS plus 0.1% BSA (10 µg/mL) and frozen in 10 µL aliquots and stored at −80°C.

Using this system, around 95% of oocytes have reached MII by the end of IVM (unpublished results). Post-maturation cumulus expansion was assessed using a scale as described in Vanderhyden *et al.* (1990). COCs were graded as 0: no expansion, 1+: the outer layer of cumulus cells expanded, 2: the outer half of cumulus expanded, 3: all layers expanded apart from corona radiata and 4+: maximum expansion of all layers of cumulus cells.

In vitro fertilisation and embryo culture

Male mice were sacrificed using cervical dislocation, and the vas deferens along with the cauda epididymis were collected in Wash Medium (Cook Medical) at 37°C, and excess tissue and fat were removed using a dissecting microscope. Spermatozoa were extracted into a culture dish containing pre-equilibrated 1 mL Fertilisation Media (Cook Medical) and incubated for 45–60 min at 37°C in 6% CO_2, 5% O_2, and 89% N_2 to allow sperm to capacitate (Lim *et al.* 2019). Spermatozoa were

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then added to the fertilisation drop, which contained the expanded COCs, and co-incubated for a further 4 h at 37°C in 6% CO_2, 5% O_2, and 89% N_2.

Following fertilisation, presumptive zygotes were washed in fertilisation medium and cultured in Cleave Media (20 µL drops per 10 embryos; Cook Medical) until day 5 at 37°C in 6% CO_2, 5% O_2, and 89% N_2. Preimplantation embryo development and morphology were determined by examining cleavage rate as a measure of fertilisation at 24 h, blastocyst formation at 72 h, and blastocyst development at 96 h (Mitchell *et al.* 2009). The experiment was replicated ten times and used a minimum of 40 COCs per group in each replicate.

Differential staining

Inner cell mass (ICM) and trophectoderm (TE) cell numbers were determined using differential staining as previously described by Handyside and Hunter (1984). Treatments were placed in 20 µL droplets, which were covered under oil and placed in a prewarmed culture dish at 37°C minimum of 1 h before staining. Blastocysts were incubated in 0.5% pronase for 2–3 min to remove the zona pellucida and then washed in protein-free 3-(N-morpholino) propanesulfonic acid (MOPS)-buffered media. Blastocysts were then transferred into 10 µL of 2,4,6-trinitrobenzenesulfonic acid and 90 µL of polyvinylpyrrolidone (PVP) and cultured at 4°C for 10 min. Blastocysts were then washed in MOPS medium and incubated in 20 µL 0.1 mg/mL anti-dinitrophenyl-BSA antibody for 10 min at 37°C, followed by a third wash in MOPS medium. Following the third wash, blastocysts were incubated in complement 50 µL propidium iodide (10 µg/mL; PI) and 50 µL guinea pig serum diluted in MOPS media at 37°C for 5 min in the darkness. Blastocysts were then transferred to 500 µL bisbenzimide in ethanol and incubated overnight at 4°C in darkness. The following day, stained blastocysts were placed in 500 µL of 100% ethanol, mounted in a drop of glycerol and gently flattened with a coverslip for cell counting. Stained blastocysts were imaged using an Olympus BX 51 microscope fitted with a mercury lamp. Bisbenzimide was excited and emitted at 338 nm and 505 nm, respectively, to visualise ICM cells and PI at 537 nm and 619 nm to visualise TE cells, and the number of ICM (blue) and TE (pink) cells were counted manually.

Blastocyst DNA damage

The histone modification antibody γH2AX (Cell Signaling Technology) was used to measure the incidence of DNA double breaks as an estimate of blastocyst DNA damage (Tan *et al.* 2016). Immunohistochemistry was done on blastocysts at 96 h post fertilisation, to determine the number of γH2AX-positive cells in the control and treated groups. Embryos were fixed in 4% paraformaldehyde and then washed for 30 min in phosphate-buffered saline (PBS) with 0.3 mg/mL polyvinyl alcohol (PVA) and permeabilized in 0.25% Triton-X (USB Corporation, OH, USA). To prevent non-specific binding, 10% blocking solution of goat serum in PBS-PVA (Jackson ImmunoResearch) was added for 1 h. Blastocysts were then incubated overnight at room temperature (RT) with primary antibody γH2AX diluted 1:200 in 10% goat serum (in PBS-PVA). No primary antibody was used as the negative control. The next day, blastocysts were washed in PBS-PVA solution 3 × 2 min prior to labelling with secondary antibody, anti-rabbit Alexa 594-conjugated secondary antibody (1:500, Life Technologies) and 4'6 dimidino-2-phenylindole (3 mM; DAPI) for a nuclear stain at RT for 2 h. Blastocysts were washed 3x in PBS and loaded onto a glass slide, covered with fluorescent mounting medium, and gently overlaid with a coverslip using a spacer. Fluorescence was detected using a Fluoview FV10i confocal laser scanning microscope (Olympus) at excitation 405 nm, emission wavelength 430–470 nm for DAPI, and excitation 594 nm, emission wavelength 610–710 nm for γH2AX-positive cells. Instrument settings were kept constant for the replicates. Images were captured at 2 µm intervals throughout the entire embryo and a z-stack projection was generated. DAPI and γH2AX images were then merged and flattened, and the number of nuclei containing γH2AX-foci counted manually. The experiment was replicated three times and used a minimum of 40 blastocysts per group in each replicate.

Vitrification/warming

Blastocysts were vitrified using the CryoLogic (Victoria, Australia) vitrification method as previously described by Frank *et al.* (2019). Briefly, four-well dishes were set up with 600 µL of HM, equilibrium solution (ES; HM supplemented with 7.5 % ethylene glycol and dimethyl sulphoxide (DMSO)), and vitrification solution (HM supplemented with 15% ethylene glycol and DMSO and 0.5 M sucrose) prewarmed to 37°C and pipetted into each well. Five blastocysts in 2 µL HM were transferred using a 3 µL Gilson mechanical pipette into HM and then transferred to ES for 3 min. Blastocysts were then transferred to the vitrification solution for 45–60 s before loading them onto the Fibreplug (CryoLogic) in 2 µL vitrification solution. Once loaded, the Fibreplug was immediately vitrified in the vapour phase of liquid nitrogen, followed by storage in a Fibreplug straw in liquid nitrogen.

For warming four well dishes containing 600 µL warming solution (WS) 1, WS 2, WS 3 (HM supplemented with 0.3, 0.25 and 0.15 M sucrose, respectively) and HM were prewarmed to 37°C. Straws were kept in liquid nitrogen, and the Fibreplug fibres were removed from the straws and quickly placed in WS 1 for 30 s, subsequently placed into WS 2, WS 3, and then HM for 5 min each. After warming, blastocysts were placed into

pre-equilibrated Cleave Media for 3–4 h to allow them to re-expand to determine survival rates.

Pseudopregnancy and embryo transfer

Swiss female mice aged 8–12 weeks were used as recipient mothers for embryo transfer. Females were placed with vasectomised adult CBAF1 males to induce pseudopregnancy. As the addition of 10 ng/mL GM-CSF to IVM media was the only dose to improve cumulus expansion, blastocyst rates and cell numbers, it was chosen for our blastocyst transfer experiments. On day 3.5 post coitum, six re-expanded blastocysts from each group were surgically transferred to contralateral uterine horns chosen at random in 17 recipient mothers as described previously (Zander-Fox *et al.* 2015). Mice were culled on day 18.5 of pregnancy, and the number of implantations, resorptions, and abnormal and normal fetuses (as an estimate of birth rate) was determined. Fetal weight, crown–rump length and diameter, and placental weight were also determined.

Statistical analysis

All data are expressed as mean ± S.E.M. unless stated otherwise. Statistical analysis was performed using IBM SPSS version 28 and GraphPad Prism version 9. A P value of < 0.05 was considered to be statistically significant and ≤ 0.1 was classified as a trend. Data were assessed for normality using the Kolmogorov–Smirnov normality test. Cumulus expansion was analysed by Poisson log-linear generalised linear models with the Bonferroni *post hoc* test. Blastocyst development data were normalised with logarithmic transformation and assessed using one-way ANOVA with Dunnett's multiple comparison *post hoc* test. Blastocyst cell counts (ICM, TE, total cell, ICM/TE ratio) and DNA damage were measured using univariate general linear models with the Bonferroni *post hoc* test. Implantation rates, fetal numbers, and fetal and placental weights were analysed using either using a binary logistic generalised linear model or a univariate general linear model with pair-wise comparison. In these models, the pseudopregnant mother was fitted as a random factor.

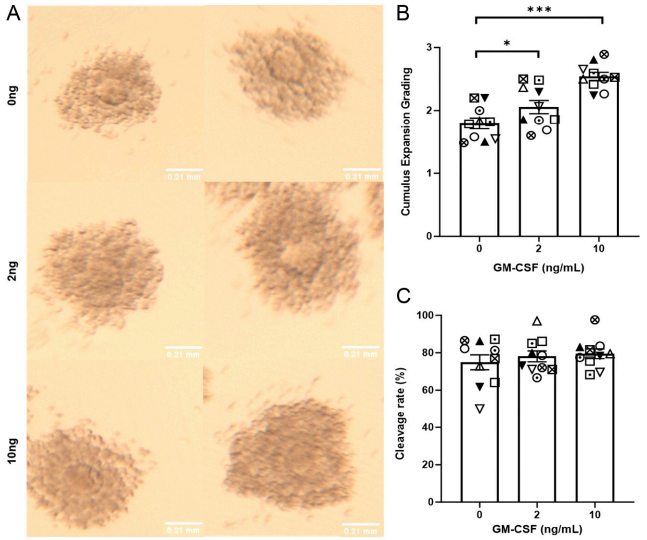

Figure 1

The effect of GM-CSF on cumulus expansion and fertilisation rates following 16 h of IVM. (A) Representative pictures of COC cumulus expansion for 0 (control), 2, and 10 ng/mL of GM-CSF, (B) the proportion of COCs at each cumulus expansion classification, and (C) proportion of 2 cells 24 h post-insemination from the total number of COCs inseminated. Values are expressed as the proportion (cumulus expansion) or mean ± S.E.M. of each replicate (fertilisation). Cumulus expansion was determined in a minimum of 400 COCs per group from 10 replicates. The fertilisation rate was determined in at least 330 zygotes per group from 10 replicates. Different symbols represent the mean of each replicate across the groups. *P < 0.05, ***P < 0.001.

Results

Effect of GM-CSF during IVM on cumulus expansion and fertilisation rates

The addition of 2 ng/mL GM-CSF increased the proportion of COCs with 1+ and 2+ cumulus expansion compared with control (+15.1%; Fig. 1A and B, P < 0.05). The addition of 10 ng/mL GM-CSF to IVM media increased the proportion of COCs with 2+ and 3+ cumulus expansion scores compared with control (+41.34%; Fig. 1A and B, P < 0.001). There was no effect of GM-CSF during IVM on subsequent cleavage rates at either concentration (Fig. 1C, P > 0.05).

Table 1 Effect of GM-CSF during IVM on embryo development. Values are expressed as a percentage of total oocytes and are the mean ± S.E.M. of ten replicates. Data are representative of a minimum of 200 blastocysts per treatment group.

GM-CSF (ng/mL)	***n*** (oocyte)	**72 h post insemination early blastocyst** (%)	**96 h post insemination total blastocyst** (%)
0	450	4.98 ± 1.71	48.88 ± 4.35
2	479	5.94 ± 2.27	48.40 ± 2.94
10	447	8.64 ± 3.27	57.24 ± 4.43*

*P < 0.05.

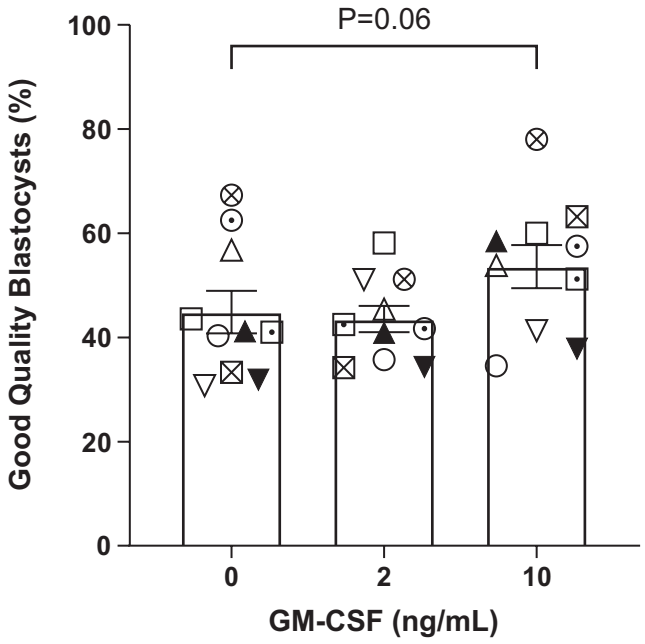

Figure 2

The effect of GM-CSF during IVM on the proportion of good-quality (expanded and hatched) blastocysts after 96 h of culture. Values are expressed as a percentage of mean ± S.E.M. of total oocytes. Blastocyst development was determined in a minimum of 200 blastocysts per group from ten replicates. Different symbols represent the mean of each replicate across the groups.

Effect of GM-CSF during IVM on blastocyst development

There was no effect of 2 ng/mL of GM-CSF during IVM on blastocyst rate or the number of hatched blastocysts compared with the control group at 96 h (Table 1 and Fig. 2). The addition of 10 ng/mL GM-CSF during IVM significantly increased the number of blastocysts present at 96 h of culture (+17.0%, Table 1, $P < 0.05$) compared with control. Overall, there was an increase in the number of good-quality blastocysts (expanded plus hatched; Fig. 2, $P < 0.05$) with a trend for an increase in the 10 ng/mL group ($P = 0.06$).

Effect of GM-CSF during IVM on blastocyst cell numbers and DNA damage

There was no effect of 2 ng/mL GM-CSF on blastocyst cell numbers or incidence of DNA damage compared with control (Fig. 3, $P > 0.05$). The addition of 10 ng/mL GM-CSF significantly increased blastocyst ICM cell number (+25.2%, Fig. 3A and D, $P < 0.001$), TE cell numbers (+29.9%, Fig. 3A and C, $P < 0.01$) and total blastocyst cell numbers (+28.6%, Fig. 3A and B, $P < 0.001$). There was no effect on the ratio of ICM/TE (Fig. 3E, $P > 0.05$) compared with control. The addition of 10 ng/mL GM-CSF to IVM media decreased the number of blastocyst cells that were positive for DNA damage (−16.2%, Fig. 3F, G, and H, $P < 0.001$) compared with control and 2 ng/mL GM-CSF groups (Fig. 3F, G, and H, $P < 0.05$).

Effect of GM-CSF during IVM on implantation rate and fetal development following blastocyst transfer

The addition of 10 ng/mL of GM-CSF during IVM had no effect on re-expansion after warming (0 ng/mL: 60.24 ± 3.15 vs 10 ng/mL: 59.31 ± 2.83). The addition of GM-CSF during IVM increased the blastocyst implantation rates (+21.0%) and fetal number (+17.0%) compared with control (Table 2, $P < 0.05$). There was no effect of GM-CSF on any fetal or placental parameters measured (Table 2, $P > 0.05$).

Table 2 Effect of GM-CSF during IVM on implantation rate and fetal development following blastocyst transfers. Values are expressed as a percentage of total oocytes and are the mean ± S.E.M. for recipients that became pregnant. A total of 100 re-expanded blastocysts from each group were transferred to contralateral horns chosen at random to 17 recipient mothers, four of whom failed to become pregnant. Fetal and placental data were determined for 21 fetuses in the control group and 38 fetuses in the GM-CSF group.

	Control	10 ng/mL GM-CSF	*P*
Implantation rate (%)	32.0 ± 0.47	53.0 ± 0.05	0.002
Fetal Number (%)	21.0 ± 0.41	38.0 ± 0.49	0.007
Number of resorptions (%)	10.50 ± 3.67	14.71 ± 4.25	NS
Fetal number/implantation rate (%)	67.0 ± 0.84	71.0 ± 0.63	NS
Fetal weight (mg)	854.02 ± 29.96	903.20 ± 24.72	NS
Crown-to-rump length (mm)	18.66 ± 0.56	18.69 ± 0.46	NS
Placental weight (mg)	128.13 ± 5.49	130.14 ± 4.53	NS
Fetal/placental weight ratio	7.06 ± 0.44	7.36 ± 0.346	NS

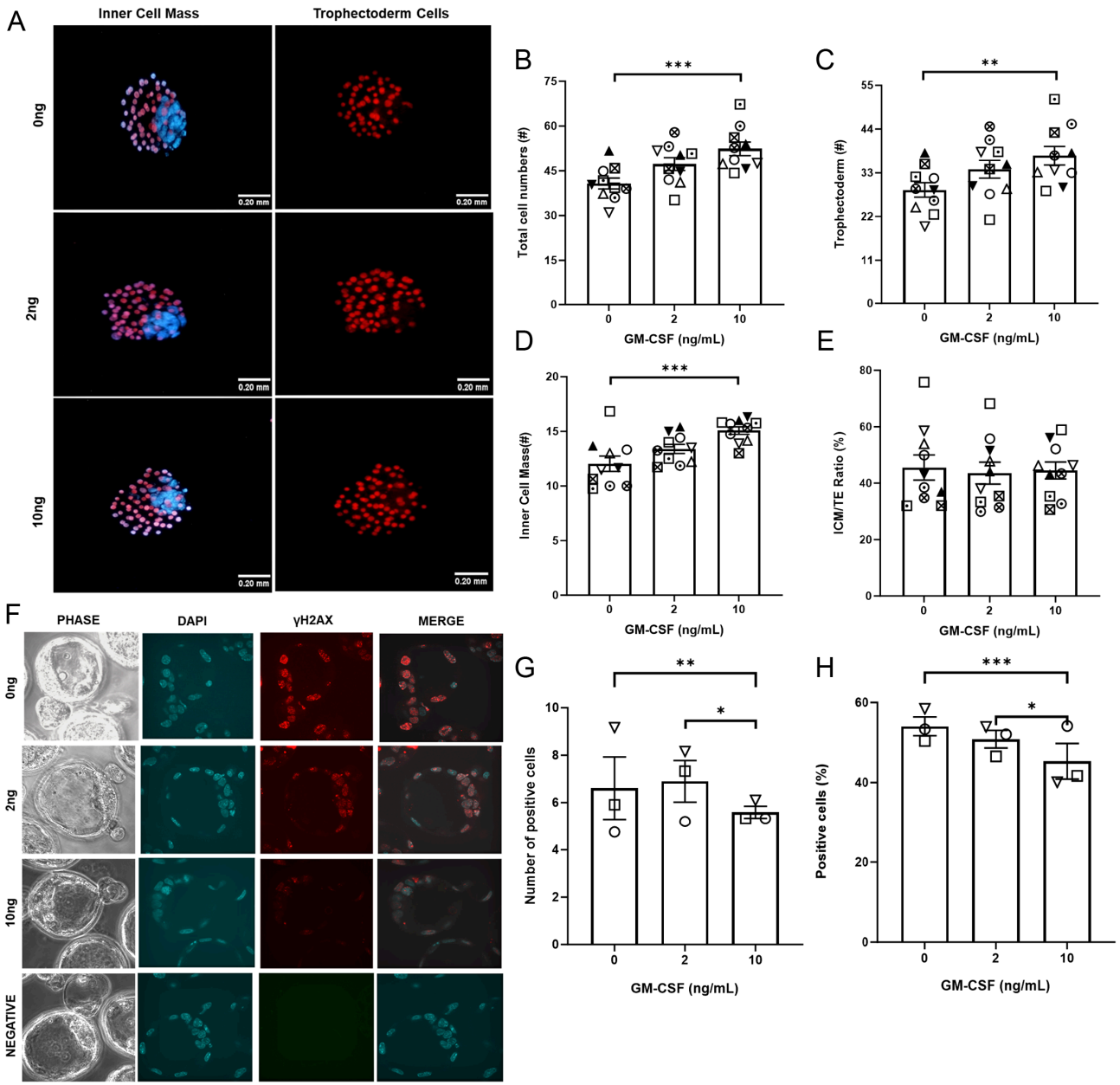

Figure 3

The effect of GM-CSF during IVM on blastocyst cell numbers and blastocyst DNA damage after 96 h of culture. (A) Representative pictures of blastocyst cell staining for 0 (control), 2, and 10 ng/mL GM-CSF groups, (B) total blastocyst cell numbers, (C) TE cell numbers, (D) ICM cell numbers, and (E) ICM/TE ratio. (F) Representative images of γH2AX staining for 0 (control), 2, and 10 ng/mL of GM-CSF. γH2AX staining (red): DAPI nucleus staining (blue). Negative control: no γH2AX antibody, (G and H) Number and percentage of positive cells for γH2AX. Values are expressed as mean ± S.E.M. Cell numbers were determined in a minimum of 65 blastocysts per group from ten replicates. DNA damage was determined in a minimum of 40 blastocysts per group from three replicates. Different symbols represent the mean of each replicate across groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Discussion

The present study was undertaken to determine whether the addition of GM-CSF during IVM could improve oocyte developmental competence, which we determined by examining preimplantation embryo development and implantation and birth rates following embryo transfer. We showed that the addition of 10 ng/mL GM-CSF during IVM increased the blastocyst rate and tended to increase the number of good-quality blastocysts. The addition of GM-CSF also increased blastocyst ICM, TE and total cell numbers. Similar effects on blastocyst cell numbers have

been reported in a variety of species when GM-CSF is added during embryo culture. In mice, Chin *et al.* (2009) showed that embryos cultured with 2 ng/mL of GM-CSF had increased blastocyst ICM and TE cell numbers, resulting in greater total cell numbers. In cattle, Loureiro *et al.* (2009) showed that GM-CSF tended to increase ICM numbers of IVP-derived blastocysts, while in humans, Sjöblom *et al.* (1999) reported that the addition of GM-CSF following fertilisation increased ICM numbers.

The addition of GM-CSF during IVM also reduced the incidence of DNA damage in blastocysts. The effect of GM-CSF during embryo culture on embryo development is thought to be mediated by the downregulation of stress and apoptotic gene expression, particularly in the ICM (Sjöblom *et al.* 2002, Chin *et al.* 2009). Whether a similar mechanism operates following the addition of GM-CSF during IVM in COCs to increase blastocyst ICM numbers remains to be determined. We also demonstrated that the addition of 10 ng/mL GM-CSF during IVM resulted in increases in implantation and birth rates following the transfer of vitrified blastocysts. Sjoblom *et al.* (2005) also reported increased birth rates for GM-CSF cultured embryos following fresh embryo transfer in mice. Previous work has shown that increases in blastocyst cell numbers, in particular ICM numbers, are associated with an increase in implantation and birth rates in mice (Lane & Gardner 1997) as well as other species. In the present study, the addition of GM-CSF during IVM also increased ICM cell numbers, which may also have been responsible for the increase in implantation rate we observed.

We saw no changes in gross fetal or placental morphology following embryo transfer. Previous studies have reported increases in fetal weights in mice when GM-CSF is added to embryo culture (Sjoblom *et al.* 2005). In addition, these workers showed that GM-CSF reduces the detrimental effect of embryo culture on placental morphogenesis, fetal viability, and post-natal growth. Similar improvements have also been shown in cattle, where the addition of GM-CSF during embryo culture partially normalised aberrant gene expression and growth that occurs as a result of IVP (Siqueira *et al.* 2017). Even though we saw no changes in gross fetal and placental morphology, it remains to be determined whether the addition of GM-CSF during IVM influences gene expression following genomic activation as well as post-natal growth.

Oocyte developmental competence has been shown previously to be a determinant of embryo development and birth outcomes in many species, which can be influenced by a variety of factors, including IVM (Richani *et al.* 2021). GM-CSF has been shown to increase glucose uptake in many non-haemopoietic cells, including blastocysts (Robertson *et al.* 2001) and spermatozoa (Tanhaye Kalate Sabz *et al.* 2022). A similar mechanism was also suggested to be responsible for the increase in cumulus expansion in cattle when GM-CSF was added during IVM (Peralta *et al.* 2013). This increase was due to an increase in cumulus cell number as the number of viable to nonviable cells remained the same. Given that we also saw an increase in cumulus expansion in our study, future work will further examine the effect of GM-CSF on glucose uptake by the cumulus cells and the supply of pyruvate and other metabolites via the gap junctions to the maturing oocyte, which is known to increase developmental competence (Richani *et al.* 2021).

In conclusion, we have shown that the addition of GM-CSF during IVM can improve oocyte developmental competence, which improves preimplantation embryo development and translates to increases in implantation and birth rates. Whether these improvements are additive to those reported previously when GM-CSF is added during embryo culture remains to be determined. Nevertheless, our findings warrant further investigation, as the addition of GM-CSF during IVM has the potential to improve livestock IVP and human IVM success rates.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the study reported.

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Author contribution statement
AS, NM, and MN conceived the study. AS performed the experiments. AS wrote the paper with contributions from NM and MN, who edited and approved the final version of the manuscript.

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