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Preclinical data support the safety of egg precursor cell repositioning using a method designed to address poor response to controlled ovarian stimulation

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Introduction

- Infertility treatment options are limited for women with poor response to controlled ovarian stimulation using hormones during *in vitro* fertilization (IVF) treatment or with diminished ovarian reserve/primary ovarian insufficiency.[1-3]
- These women struggle to have their own biological children, and IVF with egg donation or adoption are their only effective reproductive options at present.[1-3]
- OvaPrime℠ treatment is an innovative approach currently in development to address this unmet need.
- OvaPrime treatment aims to enhance a woman's egg reserve and ovarian function by isolating her own egg precursor cells from autologous ovarian cortex biopsy tissue and reintroducing them into the follicular development zone of one or both ovaries (**Figure 1**).
  - It is anticipated that reintroduced egg precursor cells may develop into eggs within newly formed follicles and increase the follicular pool.
  - This could facilitate natural conception or proliferation of a sufficient number of developmentally competent eggs in response to controlled ovarian stimulation using hormones, thereby improving live birth rates in autologous IVF cycles for women with insufficient ovarian response, among others.
- OvaPrime treatment is based on the ground-breaking discovery of egg precursor cells in the ovary and is supported by preclinical studies from numerous independent research groups.[4-8]

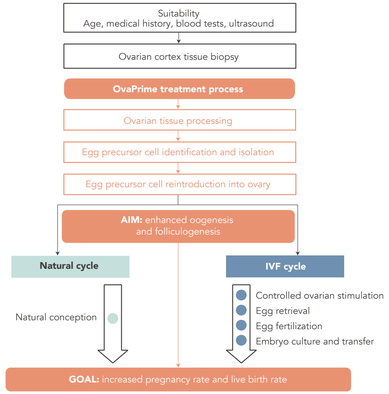

Figure 1: The OvaPrime treatment process.
IVF, *in vitro* fertilization.

Preclinical data in support of OvaPrime treatment

- Multiple independent research groups have demonstrated the existence of egg precursor cells (also called oogonial stem cells, female germline stem cells, EggPC℠ cells) in the ovarian cortex of various mammalian species, including humans.[4-10]
- Preclinical studies across multiple species have demonstrated the potential of these oocyte progenitors to increase the antral follicle pool and to generate developmentally competent eggs upon reintroduction into the ovary (**Table 1**).[4-8]

Table 1: Preclinical research in support of OvaPrime treatment

Study	Design	Outcome	Support for OvaPrime treatment
Zou et al. 2009[4]	• **Mouse:** GFP⁺ FGSCs transplanted into ovaries of chemotherapy-treated mice (infertile) • Natural mating post transplant	• GFP⁺ offspring 75–110 days post transplant • GFP gene expression found in F2 generation	Healthy live birth from egg precursor cells in mouse
Zhang et al. 2011[5]	• **Mouse:** GFP⁺ FGSCs transplanted into ovaries of chemotherapy-treated mice (infertile) • Natural mating post transplant	• GFP⁺ offspring 2 months post transplant	Healthy live birth from egg precursor cells in mouse
White et al. 2012[6]	• **Mouse:** GFP⁺ OSCs transplanted into ovaries of untreated wild-type mice (fertile) • IVF used	• GFP⁺ eggs ovulated after hormone treatment 5–6 months post transplant • Ovulated eggs fertilized *in vitro*, resulting in 35% (8/23) GFP⁺ embryos • Each mouse released ≥1 GFP⁺ egg at ovulation	Production of mature and fertilizable eggs from egg precursor cells in mouse
	• **Human:** GFP⁺ OSCs transplanted into human ovarian cortical strips, xenografted subcutaneously into immunocompromised mice • No hormonal stimulation	• OSCs matured into eggs 7–14 days post transplant into immunodeficient female mice • 15–21 GFP⁺ eggs per analyzed tissue graft	Production of immature human eggs in xenotransplanted model
Zhou et al. 2014[9]	• **Rat:** GFP⁺ or *fat-1*⁺ FGSCs transplanted into ovaries of chemotherapy-treated rats (infertile) • Natural mating post transplant	• GFP⁺ or *fat-1*⁺ offspring produced 2–5 months post transplant • GFP gene expression found in F2 generation	Healthy live birth from egg precursor cells in rat
Wolff et al. 2014[7]	• **Nonhuman primate (rhesus macaque):** autologous GFP⁺ OSCs transplanted into an ovary of an untreated monkey (fertile) • IVF used	• 1/5 eggs aspirated *in vivo* after hormone treatment was a GFP⁺ metaphase II egg	Production of a mature egg from autologous egg precursor cells in nonhuman primate

fat-1⁺: genetically modified to carry the *Caenorhabditis elegans fat-1* gene that encodes an n-3 fatty acid desaturase; FGSC, female germline stem cell; GFP, green fluorescent protein (used as a fluorescent marker to track egg progenitor cells *in vivo*); GFP⁺, green fluorescent protein positive; IVF, *in vitro* fertilization; OSC, oogonial stem cell.

Objective

- To test the safety of autologous egg precursor cell reintroduction into the ovaries using the OvaPrime treatment method in nonhuman primates, thus strengthening preclinical safety evidence supporting OvaPrime.

Methods

- This Good Laboratory Practice (GLP)–level toxicology study performed by WIL Research (Ashland, OH, USA)* evaluated the safety of an egg precursor cell reintroduction technique in nonhuman primates; the design is summarized in **Figure 2**.
- 18 healthy, nulliparous, nonpregnant female cynomolgus monkeys (*Macaca fascicularis*) 3–4 years of age were evaluated.
- After removal of the right ovary from each animal via abdominal laparotomy, ovarian cortex tissue was mechanically and enzymatically digested, and egg precursor cells were isolated via fluorescence-activated cell sorting using a proprietary antibody against DEAD box protein 4 (*DDX4*) and cryopreserved by slow freezing.
- 2 weeks after ovarian removal, up to 100% of the autologous egg precursor cells isolated from an animal were surgically reintroduced to the cortex of the contralateral ovary of the same animal via midline laparotomy using a 0.5-mL (1/2 cc) tuberculin syringe and 28-gauge 1/2-inch needle, injecting a total volume of ~100 µL in a series of 3–5 injections.
  - The animals were split into 3 groups: 6 animals were administered the vehicle (phosphate-buffered saline), 6 animals 10% of the isolated egg precursor cells, and 6 animals 100% of the isolated egg precursor cells.
  - Based on interspecies allometric scaling between cynomolgus monkeys and humans, the 10% dose may act as a reasonable proxy for the amount of tissue (and by extrapolation, the relative number of egg precursor cells) that would be used in the clinical setting, while the 100% dose may be considered a 10X safety margin.
- 3 animals per group were assigned to an interim necropsy at 3 months after egg precursor cell reintroduction, while the remaining 3 animals per group were assigned to the final necropsy at 6 months after egg precursor cell reintroduction.
  - Necropsies were performed. The ovaries, reproductive organs, and other selected organs and tissues were collected for weighing and microscopic examination.
- Animals were assessed twice daily for morbidity and mortality following egg precursor cell reintroduction until the interim or final necropsies.
- Additional clinical evaluations included: menstrual cycle assessment by vaginal swabbing, body weight, hematology, serum chemistry, serum hormone analysis for estradiol and progesterone, and urinalysis.
  - Sampling frequency varied by parameter (e.g., serum hormones sampled daily during certain phases of menstrual cycle, weight measured weekly, blood for clinical pathology parameters taken monthly).

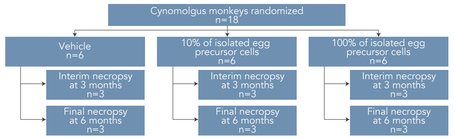

Figure 2: Study design.

Results

- Egg precursor cells were successfully isolated from the right ovaries of the female cynomolgus monkeys. Isolated egg precursor cells from vehicle control animals had 66.7–81.6% cell viability; this assessment of the control egg precursor cell viability was used as an estimate of the overall number of live egg precursor cells reintroduced.
- Following injection of up to 100% of isolated egg precursor cells into the left ovary, all animals survived to the scheduled necropsy and no morbidity or treatment-related abnormalities were observed.
- Physical observations and laboratory evaluations were normal at 6 months post transplant.
  - Mean body weight did not differ between the 10% and 100% egg precursor cells groups and the vehicle control group in the 6 months after egg precursor cell reintroduction (**Figure 3**).
  - No significant changes were observed in hematology, serum chemistry or urinalysis parameters (data not shown).

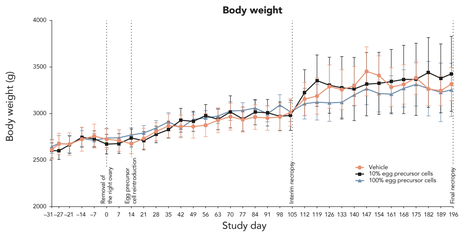

Figure 3: Body weight after egg precursor cell reintroduction.
Mean±SEM body weight before and after egg precursor cell reintroduction.

- At necropsy, no differences in ovarian histology were observed between the 2 treatment groups and the vehicle control group (**Figure 4**).

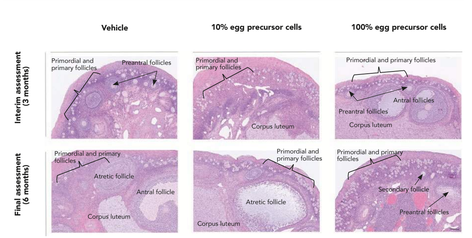

Figure 4: Representative ovarian sections stained with hematoxylin and eosin from the interim and final necropsies.
Representative hematoxylin-eosin–stained ovarian sections from the interim and final necropsies at 3 and 6 months, respectively, after egg precursor cell reintroduction showing no obvious histologic abnormalities.

- All animals exhibited normal menstrual cycles after surgical reintroduction of egg precursor cells, with no significant differences in estradiol and progesterone hormone levels (data not shown) or menstrual cycle length (**Table 2**) between the 2 treatment groups and the vehicle control group.

Table 2: Menstrual cycle length after egg precursor cell reintroduction

	Vehicle	10% egg precursor cells	100% egg precursor cells
Mean±SD cycle length in days	32±2.8	33±2.9	31±3.4

- Ovarian follicular distribution was comparable between the 2 treatment groups and the vehicle control group 6 months after egg precursor cell reintroduction, suggesting that there was no disruption to normal ovarian physiology (**Table 3**).

Table 3: Ovarian follicular distribution at the final necropsy (representative assessment)[a]

Group	Vehicle			10% egg precursor cells			100% egg precursor cells		
Animal	I	II	III	I	II	III	I	II	III
Atretic follicles	H	H	H	H	M	M	H	M	H
Primary/primordial follicles	H	H	L	M	H	M	M	M	H
Secondary follicles	M	M	L	M	M	L	L	L	L
Tertiary follicles	H	H	H	H	H	L	M	L	H
Luteal bodies	M	L	M	M	M	M	M	M	M

[a]Follicular distribution per representative assessment in the 3 animals per group that were necropsied 6 months after egg precursor cell reintroduction. For each animal, sections throughout the entire ovary were reviewed and follicle numbers were categorized as low, moderate, or high based on a comparative analysis between groups.
H, high; L, low; M, moderate.

Conclusions

- Physiological studies across multiple species provide evidence for the capacity of isolated and reintroduced egg precursor cells to develop into functional eggs when reintroduced into the follicular development zone of the ovary.[4-8]
- This GLP toxicology study demonstrates the safety of egg precursor cell reintroduction using the OvaPrime treatment approach in nonhuman primates (cynomolgus monkeys).
  - Surgical reintroduction into the left ovary of up to 100% of the autologous egg precursor cells isolated from the right ovary was well tolerated, and no morbidity or mortality was observed. All animals survived to the scheduled necropsy.
  - There were no treatment-related macroscopic findings, histological abnormalities or alterations in body weight, hematology, serum chemistry or urinalysis.
  - Reintroduction of egg precursor cells did not affect hormonal or menstrual cycles.
- These nonhuman primate safety data add to the preclinical evidence supporting the feasibility and safety of OvaPrime treatment, and thereby support the future clinical evaluation of OvaPrime treatment as a therapeutic approach to address infertility in women with low/diminished or no egg reserve, insufficient ovarian function or failed follicular response to controlled ovarian stimulation using hormones.

Limitations

- This was a safety study only. Egg precursor cell biologic activity after reintroduction, and matings and pregnancy rates, were not studied. Thus, the effectiveness of OvaPrime treatment (live birth rates) was not included in this study.

Disclosures

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*WIL Research is now known as WIL Research, a Charles River Company.

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