Clinical Significance of Expression of Stem Cell Markers in Human Ovarian Luteinized Granulosa Cells during Assisted Reproduction Technologies

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Abstract

The adult human ovary is composed of various cell types. Preovulatory follicles contain two distinct types of granulosa cells that arise during folliculogenesis as the cell populations segregate upon formation of the fluid-filled follicular antrum. The granulosa cells line the follicle wall, reside very close to the basement membrane and are essential for estrogen production and follicular rupture. The cumulus cells are closely connected to the oocyte through a gap junction network and are associated with the oocyte development. Paracrine interactions between ovarian somatic cells and germ cells are critical for normal follicular development and oocytes also promote granulosa cell proliferation and differentiation. There are strong evidences that a subpopulation of granulosa ovarian cells have pluripotent and self-renewal capabilities. The presence of stem cell markers in the ovarian luteinized granulosa cells of women undergoing assisted reproduction technologies is an important subject for studies in terms of their possible regenerative role and their possible prognostic significance for the infertile citizens. Expression of the stem cell marker Oct-4 in human ovarian luteinized granulosa cells from women undergoing IVF or ICSI indicates the presence of stem cells in these cells. The absence of DAZL gene expression in these cells indicates that the stem cells found in granulosa cells cannot be differentiated in germ cells. Also, a clinical significance for the number of oocytes retrieved and the expression levels of Oct-4 in human luteinized ovarian granulosa cells is possible to exist. More specifically, the expression of Oct-4 mRNA in granulosa cells appears to play an important role in the regulation of follicular growth during assisted reproduction technologies.

Keywords: Oct-4; Stem cells; Markers; Ovary; Granulosa cells

Abbreviations: ART: Assisted Reproduction Technology; OCT-4: Octamer-Binding Transcription Factor 4; DAZL: Deleted In Azospermia-Like; IVF: In vitro Fertilization; ICSI: Intracytoplasmic Sperm Injection; GDF-9: Growth Differentiation Factor 9; BMP-15: Bone Morphogenetic Protein 15; FSH: Follicle-Stimulating Hormone; ESCs: Embryonic Stem Cells; VSELs: Very Small Embryonic-Like Stem Cells; SSEA: Stage Specific Embryonic Antigen-(SSEA)-3/4 (human), Sca-1 and Stem Cells (VSELs) with pluripotent potential and positive for Oct-4, self-renewal of undifferentiated embryonic stem cells and is therefore used as a marker of Embryonic Stem Cells (ESCs) [7,15]. In addition, it has been found that a minor population of Very Small Embryonic-Like Stem Cells (VSELs) with pluripotent potential and positive for Oct-4, Stage Specific Embryonic Antigen-(SSEA)-3/4 (human), Sca-1 and NANOG are present in the bone marrow, cord blood, epidermis, heart, pancreas, testis, bronchial epithelium and ovaries [9,16-18]. These results suggest that isolation Oct-4 positive VSELs may serve as a good source of pluripotent stem cells in adult tissues and have a potential application in regenerative medicine [9,18].

Octamer-Binding Transcription Factor-4 (OCT-4, also known as POU5F1) is a member of Pit-Oct-Unc (POU) transcription factor family and is known to play a precise role in the maintenance of self-renewal and pluripotency in Embryonic Stem Cells (ESCs) [7-9]. OCT4 is expressed in unfertilized oocytes, the Inner Cell Mass (ICM) of the blastocyst, embryonic stem (ES) cells and germ-cell tumors [9]. Oct-4 works with other transcription factors (e.g. FOXD3, SOX2, STAT3) in a cooperative fashion to regulate many genes via the consensus motif ATGCCAAT [10-13]. Oct-4 gene contains the Proximal Enhancer (PE) and the distal enhancer (DE) that are important for Oct-4 cell type-specific expression, and the four conserved domains CR1-CR4, that are important for Oct-4 basal expression [14]. Oct-4 expression is restricted to pluripotent cells, while the loss of OCT-4 expression may be associated with loss of pluripotentiality [12]. OCT-4 is involved in the self-renewal of undifferentiated embryonic stem cells and is therefore used as a marker of Embryonic Stem Cells (ESCs) [7,15]. In addition, it has been found that a minor population of Very Small Embryonic-Like Stem Cells (VSELs) with pluripotent potential and positive for Oct-4, Stage Specific Embryonic Antigen-(SSEA)-3/4 (human), Sca-1 and NANOG are present in the bone marrow, cord blood, epidermis, heart, pancreas, testis, bronchial epithelium and ovaries [9,16-18]. These results suggest that isolation Oct-4 positive VSELs may serve as a good source of pluripotent stem cells in adult tissues and have a potential application in regenerative medicine [9,18].

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The protein DAZL (DAZ-like) is RNA binding protein which is a member of the DAZL (deleted in azoospermia like) family which also includes BOULE and DAZ. The genes of the DAZL family encode proteins with a highly conserved RNA-binding motif (RNA Recognition Motif, RRM) and a unique DAZ repeat of 24 amino acids. These proteins are believed to function in the posttranscriptional regulation of messenger RNA (mRNA) expression [19,20]. The proteins of the DAZL family are located to nucleus and cytoplasm of the fetal germ cells [21]. In males, DAZL is expressed during spermatogenesis in gonocytes, spermatogonia and primary spermatocytes. During meiosis, DAZL is translocated from the nucleus of the spermatogonios into the cytoplasm of secondary spermatocytes, spermatids and spermatozoa [22-25]. During oogenesis, human DAZL is expressed in the cytoplasm of oogonia and in developing follicular oocytes in fetal and adult ovaries [26-30]. In human males, decreased DAZL expression has been reported in testes which produce little or no sperm [31] and in human females may be associated with primary amenorrhea or premature ovarian failure [26,32]. DAZL has also been reported to be expressed in human and mouse granulosa cells [24,25], human theca interna cells [27] and in the granulosa-luteal cells of human corpus lutea [33,34], but this remains controversial [35]. In addition, DAZL and Oct-4 gene expression has been found in human amniotic fluid cells suggesting the potential of these cells as a multipotent cell course for regenerative somatic cell therapy [21].

In adult human ovaries, the ovarian surface epithelium is a source of germ cells. Bukovsky et al. [36] reported that granulosa cell nests migrate through the dense ovarian stroma to the deep cortex to contribute to the follicular renewal [36]. Granulosa cell nests were not observed in climacteric ovaries and in women with Premature Ovarian Failure (POF) or in postmenopausal ovaries [37,38]. Virant-Klun et al. found stem cells in the adult human ovaries that develop into oocyte-like and parthenote-like structures [39]. Also Parte et al. detected pluripotent gene transcripts of Oct-4, NANOG, Sox-2, TERT and STAT-3 in ovarian surface epithelium, while germ cell markers like c-Kit, DAZL, GDF-9, VASA and ZP4 were localized in oocyte-like structures [40]. Stem cells in the ovarian surface epithelium are an important subject for further studies in terms of their possible regenerative role (potential oogenesis in vitro and differentiation into different types of somatic cells for regenerative medicine) and therapeutics in patients with aggressive ovarian epithelial cancers [41]. Kossowska-Tomaszczyk et al. [42] demonstrated the presence of multipotent granulosa cells, which survived in the presence of Leukemia-Inhibiting Factor (LIF) [43]. The researchers in order to get past the fact that the status of infertility clinical background and the assisted reproduction outcomes and we think this has prompted the search for additional parameters that can support morphological and metabolic evaluations of the oocyte in order to appropriately select those that have the greater chance of fertilization and development.

In this respect, the analysis of granulosa cells is a good approach for providing such supplementary information. We investigated for the first time the correlations between the presence or absence and the levels of Oct-4 gene expression in granulosa cells with infertility clinical and gene expression patterns of the oocyte and the follicular micro-environment in which the oocyte grows and matures [4]. In view of concept of the beneficial effect of granulosa cells on oocyte maturation and the need for independent prognostic markers of better outcomes with conventional IVF for couples with non-male factor infertility, studies were focused on target genes in luteinized granulosa cells of the human ovaries. In fact, there are no morphological or physiological features of oocytes that can predict whether IVF fertilization will be successful, or whether is a need for ICSI unrelated to male factor infertility. Moreover, in some countries, not all retrieved oocytes can be fertilized due to legal limitations [46]. In such situations, predicting embryo quality is even more challenging because the time when the predictive evaluation can be performed is limited to the interval between oocyte retrieval and fertilization. This has prompted the search for additional parameters that can support morphological and metabolic evaluations of the oocyte in order to appropriately select those that have the greater chance of fertilization and development.
granulosa cells from each patient separately in correlation with duration of ovulation induction, number of follicles aspirated, number of oocytes retrieved, number of mature oocytes retrieved, embryo grade and clinical pregnancy and found a clear clinical significance only for the number of oocytes retrieved suggesting that Oct-4 expression positively affects the oocyte development during ART. The possibility for stem cell contamination during egg retrieval or granulosa cells collection and possible Oct-4 expression should be excluded because of the absence of DAZL gene expression, which is typically expressed in gametes. Our population included only patients with male or tubal factor infertility. Studying any clinical significance in ART of the expression of stem cell markers in luteinized granulosa cells is a new field of knowledge and according to our knowledge the present study is the first one. Previous studies on the same field were not done before. More studies were performed to correlate the impact of apoptosis or survival factors in granulosa cells with ART parameters and outcome. The clinical significance of the expressions of apoptotic factors in granulosa cells during art is controversial [47,48]. However, the expression of survival factors in granulosa cells gives some promises. Greene et al. [49] found that IGF1, IGF2 and their receptors are down regulated in ovarian granulosa cells of women with Diminished Ovarian Reserve (DOR) compared to those with Normal Ovarian Reserve (NOR) undergoing In vitro Fertilization (IVF) [49]. Also, Fujino et al. [50] studied the expression of survivin gene in granulosa cells from infertile Japanese patients and found that the gene expression levels of survivin in patients with endometriosis were significantly lower than in patients with male factor infertility. The gene expression levels of survivin in total pregnant patients were higher than those in total non pregnant patients [51]. Moreover, we studied only normal women (male factor infertility) and women with tubal factor infertility who underwent IVF or ICSI and embryo transfer [51]. Women with endometriosis or polycystic ovarian syndrome were not included in our study since endometriosis and androgens promote apoptosis [44,52]. We found a statistically significant increased expression of survivin in granulosa cells of women who had tubal factor infertility compared to normal women (male factor infertility). Therefore, it seems that survivin acts a protective role in the ovarian micro-environment. It is possible that survivin might try to protect ovaries, with possible influenced perfusion due to ipsilateral salpingectomy. In cases with tubal inflammation or hydrosalpinges survivin might try to protect the ovaries from follicular apoptosis in a paracrine environment [51].

In conclusion, a clear clinical significance for the number of oocytes retrieved and the expression levels of the stem cell marker Oct-4 in human luteinized ovarian granulosa cells is possible to exist. More specifically, the expression of Oct-4 mRNA in granulosa cells appears to play an important role in the regulation of follicular growth during ART. It would be interesting if further studies investigated any clinical significance of Oct-4 gene expression in granulosa cells of patients with Diminished Ovarian Reserve (DOR) as such population was not included in our study [43]. Also, it would be interesting if more studies validated the expression of Oct-4 using Western blot analysis and immune-fluorescence on granulosa cells in order to overcome possible limitations of our study and reinforce our findings [43].

References


