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Original article

Oct-4 is highly expressed in stem/progenitor cells and in primordial follicles of the fetal human ovary

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Abstract

Oct-4 (Octamer-binding transcription factor 4) is a member of the POU (Pit-Oct-Unc) family. During development, Oct-4 is expressed in embryonic stem cells and in germ cell precursors. In this study, we investigated the expression of Oct-4 in the ovaries of human fetuses during gestation. The ovaries of 14 human fetuses and newborns, ranging in gestational age from 12 up to 38 weeks of gestation, were formalin-fixed, routinely processed and paraffin-embedded. Paraffin sections were immunostained with an anti-Oct-4 commercial antibody. Oct-4 expression was demonstrated in all the ovaries analyzed. Immunoreactivity for Oct-4 was detected in multiple stem/progenitor cells, including oogonia. Moreover, Oct-4 was expressed in oocytes, in primordial follicles. In ovarian stem/progenitor cells, Oct-4 was expressed in the nucleus, whereas in oocytes reactivity for Oct-4 was restricted to the cytoplasm. In the initial stages of gestation, the majority of Oct-4-positive precursor cells were detected in the external cortex. These preliminary data indicate Oct-4 as a major player in germ cell differentiation in the human ovary and as a useful marker for ovarian stem/progenitor cells. Given the ability of Oct-4 for the detection of ovarian stem/progenitor cells, further studies are needed in order to verify its ability to detect stem cells in adult ovaries.

Keywords

Oct-4, fetal ovary, development, immunohistochemistry, primordial follicles, ovarian stem cells.

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Introduction

Oct-4 (Octamer-binding transcription factor 4), also known as Oct-3 or POU5F1 (POU domain, class 5, transcription factor 1), is a transcription factor of the POU (Pit-Oct-Unc) family [1-3]. It is critically involved in self-renewal of undifferentiated embryonic stem cells [4, 5]. The activity of Oct-4 is essential for the identity of the pluripotential founder cell population in mammalian embryos [6]. In physiology, a major role has been assigned to Oct-4 in the maintenance of pluripotency in embryonic stem cells and primordial germ cells, whose survival might depend on the hypoxia-inducible factor 2α (HIF-2α) [7]. In the initial phases of embryogenesis, Oct-4 is expressed during cleavage stages and is essential for the differentiation of the blastocyst, in which Oct-4 expression is restricted to the inner cell mass and to epiblasts. After gastrulation, Oct-4 is active only in germ cells, whereas it is progressively silenced in somatic cells [8]. Oct-4 plays a fundamental role in embryonic stem cells specification toward a cardiac lineage and in mesodermal commitment of the embryonic epiblast [9].

Regarding the development of the ovary, Oct-4 regulates the pluripotency of stem cells and has an important role during oocyte growth. Studies carried out in mice demonstrated that Oct-4 plays a pivotal role in the establishment of the oocyte's developmental competence, by regulating the expression of Stella and Foxj2 at the Nanog locus [10]. Moreover, Oct-4 gene expression levels are affected by the cyclic pattern of the estrous cycle [11]. In ovarian follicles, Oct-4 has been reported to be expressed even in granulosa cells, suggesting that these cells might maintain stemness and transdifferentiation potential [12]. *In-vitro* studies on ovarian stem cells revealed immunostaining for Oct-4 in two distinct populations of stem cells, including very small embryonic-like cells and ovarian germ stem cells [13].

In human pathology, Oct-4 immunohistochemistry has been introduced in clinical practice for establishing the germ cell origin of metastatic tumors of unknown origin [14]. The knowledge that Oct-4 is highly sensitive and specific for pluripotent testicular germ cell tumors has been utilized to detect seminoma cells in lymph node metastases [15].

Given the scarcity of studies on Oct-4 expression in the fetal ovary, this study was aimed at analyzing the expression of Oct-4 in the human ovaries during intrauterine development, at different stages of development, in order to identify the ovarian cells in which Oct-4 is mainly expressed during gestation.

Patients and methods

All the procedures performed for this work were approved by the Ethics Human Studies Committee of the University Medical Center of Cagliari (according to the instructions of the Helsinki Declaration).

Fourteen female fetuses and newborns, of gestational age ranging from 12 up to 38 weeks, were analyzed in this study. According to their gestational age, patients were grouped into the following categories [16]: miscarriages (< 22 weeks), early fetal deaths (22-28 weeks), late fetal deaths (28 weeks - birth) and postnatal deaths (after birth). The main clinical data of fetuses and newborns are reported in **Tab. 1**. In each fetus and newborn, both ovaries were obtained at autopsy, fixed in 10% formalin, routinely processed and included in paraffin. Five micron-thick tissue sections were stained with H&E for histology. Tissue sections were immunostained with antibodies against Oct-4 (mouse monoclonal antibody), Cell Marque™, clone Oct-4 (MRQ-10). For immunohistochemistry, the ultraView Universal DAB Detection Kit (Ventana Medical System) protocol was used. In brief, paraffin sections were incubated for 30 minutes at 36°C with the anti-Oct-4 antibody, following the retailer's instructions.

Table 1. Clinical data.

Case n°	Gestational age (weeks)	Postnatal age	Body weight (g)	Cause of death
Miscarriages (< 22 weeks)				
1	12	-	13	Voluntary interruption of pregnancy
2	12^{+3}	-	14	Voluntary interruption of pregnancy
3	18^{+5}	-	300	Therapeutic abortion (due to maternal psychiatric disorder)
4	19	-	320	Intrauterine fetal death (due to early uterine contractions)
5	20	-	474	Intrauterine fetal death (due to diffuse renal hemorrhages)
6	21	-	270	Therapeutic abortion (due to severe intrauterine growth restriction with bone malformations)
Early fetal deaths (22-28 weeks)				
7	22	-	400	Intrauterine fetal death (multi-organ failure, asphyxia)
8	26^{+5}	-	1,410	Intrauterine fetal death (due to severe asphyxia)
9	29	-	892	Therapeutic abortion (due to complex congenital cardiomyopathy)
Late fetal deaths (28 weeks - birth)				
10	30	-	1,121	Intrauterine acute asphyxia
11	34	-	2,100	Multi-organ dysfunction following fatal *Listeria monocytogenes* sepsis
12	38^{+2}	-	2,220	Intrauterine asphyxia (true umbilical cord knot)
Postnatal death (after birth)				
13	Born at term	5 days	3,400	Disseminated Group B *Streptococcus* sepsis
14	Preterm	2 months	2,623	Sepsis (unknown agent), acute pneumonia, myocarditis, disseminated intravascular coagulopathy, multi-organ failure syndrome

Results

Oct-4 was expressed in all the fetal ovaries analyzed in this study. Immunoreactivity for Oct-4 was detected at all the gestational ages analyzed. Significant inter-individual changes were observed regarding Oct-4 expression in the different cell types concurring to ovarian organogenesis, and the nuclear or cytoplasmic localization.

At 19 weeks, immunostaining for Oct-4 was observed in all ovarian compartments, being restricted to the nuclei of stem/progenitor cells. The highest levels of immunoreactivity for Oct-4 were observed in the superficial cortex, being expressed in the nuclei of stem/progenitor cells residing in the ovarian surface (**Fig. 1**). No reactivity for Oct-4 was found, at this gestational age, in the superficial epithelium, but occasionally Oct-4-positive cells were found in between the superficial epithelium (**Fig. 1**). Oct-4-positive pluripotent ovarian cells were not homogeneously distributed in the outer cortex. Their distribution was patchy. In the outer cortex, Oct-4-expressing cells were arranged in small groups, each putatively representing a stem cell niche. Inside each group of ovarian progenitors, cells of different shapes and sizes were easily identified, including very small cells, suggesting the presence of multiple cell types inside each ovarian stem cell niche (**Fig. 2**).

At this gestational age, Oct-4-reactive stem cells, putatively representing the primordial reserve of ovarian stem cells, were subdivided into three subtypes, according to their nuclear size:

1. small-sized pluripotent cells;
2. very small embryonic-like cells (2-4 micron in diameter);
3. large progenitors, whose nucleus was at least two times in diameter compared with very small embryonic-like cells. In all ovarian stem/progenitor cells, Oct-4 was highly expressed in the nucleus. In the large cells and in the very small cells, Oct-4 was also expressed at low levels in the cytoplasm (**Fig. 1** and **Fig. 2**). Emerging from the sub-capsular zone, Oct-4-positive pluripotent

cells appeared to spread toward the inner ovarian region, where pluripotent cells of different subtype (large, small, very small) were observed scattered among negative cells. Moreover, at this gestational age, Oct-4 was expressed in primordial follicles, emerging in the deep ovarian cortex at the border with the medullary zone. In primordial follicles, Oct-4 expression was restricted to the cytoplasm of oocytes, in the absence of any significant nuclear reactivity (**Fig. 3**).

No immunoreactivity for Oct-4 was found in the flat developing granulosa cells surrounding the primordial follicles.

At 22 weeks, the pattern of Oct-4 expression was completely different. The number of pluripotent cells identified thanks to their strong nuclear expression of Oct-4 was significantly decreased in all ovarian zones, as compared to the ovaries of 19 weeks. No immunostaining was detected, at this gestational age, in the surface epithelium, nor in stem/progenitor cells localized in the outer cortex. Only scattered Oct-4-positive cells were identified in the ovarian stroma. In the inner cortex, the number of primary follicles increased, due to the progressive differentiation of primordial germ cells into oogonia. In the primordial follicles, Oct-4 was restricted to the cytoplasm of developing oocytes. In some of them, immunoreactivity was strong, whereas in others Oct-4 was mildly expressed (**Fig. 4**). No reactivity was found in flattened granulosa

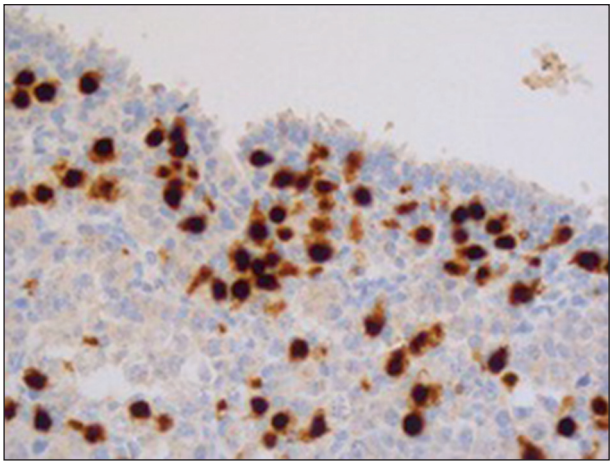

Figure 1. Ovary of a 19-week-old fetus. Strong nuclear reactivity for Oct-4 in ovarian stem/progenitor cells. Oct-4 reactive cells are concentrated in the outer cortex of the developing ovary and in between the superficial epithelium; 20x.

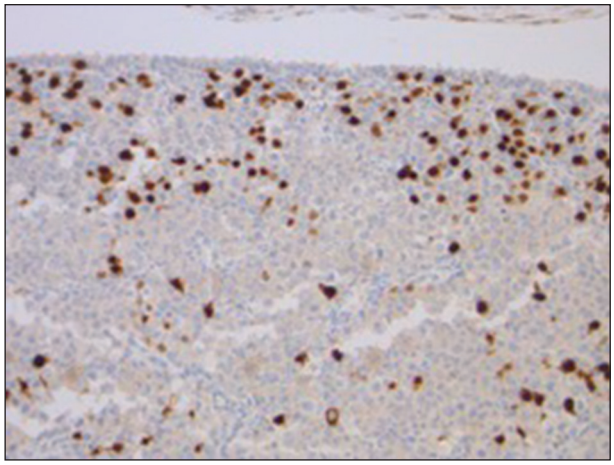

Figure 2. Ovary of a 19-week-old fetus. Oct-4 immunoreactive cells are arranged in small groups in the outer cortex, each of them putatively representing a stem cell niche. Immunoreactivity for Oct-4 is strong and restricted to nuclei; 10x.

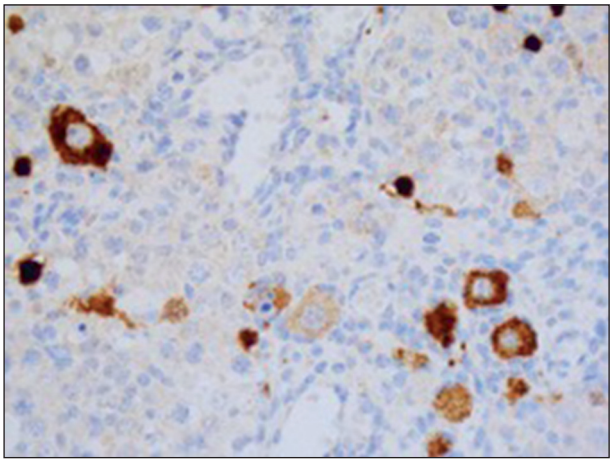

Figure 3. Ovary of a 19-week-old fetus. In the deep ovarian cortex, Oct-4 is expressed in the cytoplasm of oocytes in primordial follicles. Immunostaining is strong in the majority of oocytes; 20x.

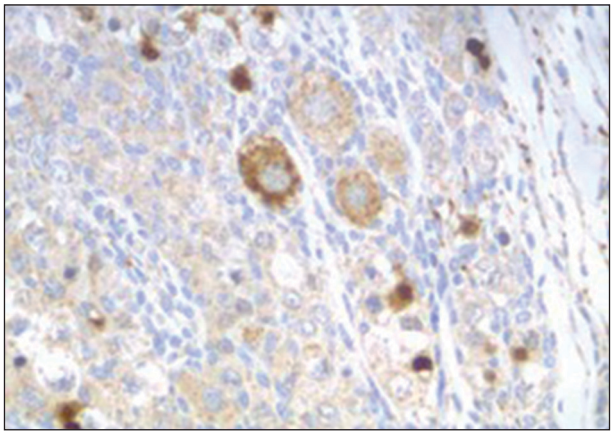

Figure 4. Ovary of a 22-week-old fetus. Variability of immunostaining for Oct-4 in developing oocytes. Oct-4 is expressed in the cytoplasm of oocytes and in the nucleus of scattered progenitor cells. Immunoreactivity is weak and restricted to the cytoplasm; 20x.

cells surrounding oocytes. Intermingled with the primary follicles, scattered few progenitor cells of different sizes were found to express Oct-4 at nuclear level (**Fig. 4**). At this gestational age, the majority of ovarian cells did not express Oct-4.

At 26 weeks, no immunostaining for Oct-4 was observed in the surface epithelium. In the outer cortex, only a few scattered progenitor cells showed a weak nuclear staining. No reactivity was detected in the ovarian stromal cells. The expression of Oct-4 was mainly observed in the oogonia of primary follicles in the inner cortex. No reactivity for Oct-4 was detected in granulosa cells. Even at this gestational age, the degree of immunoreactivity changed significantly from one follicle to the next, in spite of the absence of significant differences in morphological features among them (**Fig. 5**).

At 34 weeks of gestation, the surface epithelium did not show any reactivity for Oct-4. In the outer cortex, few scattered progenitors showed nuclear reactivity for Oct-4. In the deeper cortex, the expression of Oct-4 in the cytoplasm of oocytes was higher when compared to the previous gestational ages. Even at this gestational age, marked differences were observed regarding the degree of immunostaining, ranging from primordial follicles with strong immunoreactivity for Oct-4 to adjacent follicles with mild granular reactivity (**Fig. 6**). Oct-4 was not expressed in granulosa cells surrounding oocytes, nor in the nuclei of interstitial cells.

In the ovaries of at-term babies (38 weeks), Oct-4 expression changed significantly, according to the different degree of follicular development. No staining for Oct-4 was found in the superficial epithelium. In the outer cortex, nuclear reactivity for Oct-4 was observed in a few scattered progenitors. No immunostaining was found in the superficial and deep ovarian stromal cells. In the deep cortex, Oct-4 was expressed in the cytoplasm of the great majority of primordial follicles, with marked differences regarding the degree of immunoreactivity. On the contrary, Oct-4 was very mildly expressed in primary follicles, characterized by a larger diameter of the oocytes surrounded by one layer of cuboidal granulosa cells (**Fig. 7**).

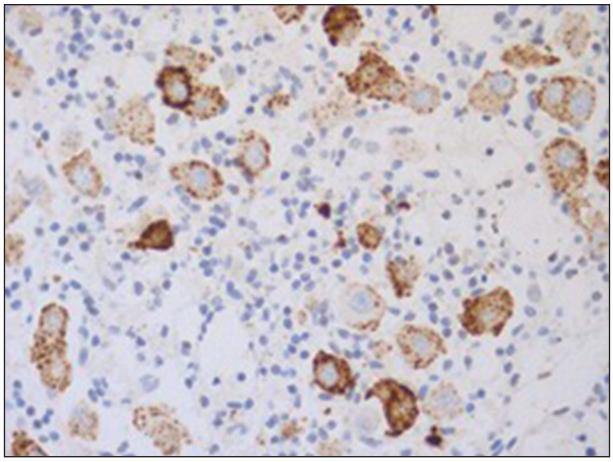

Figure 5. Ovary of a 26-week-old fetus. Oct-4 cytoplasmic immunoreactivity is granular, strong and restricted to the oogonia of primary follicles; 20x.

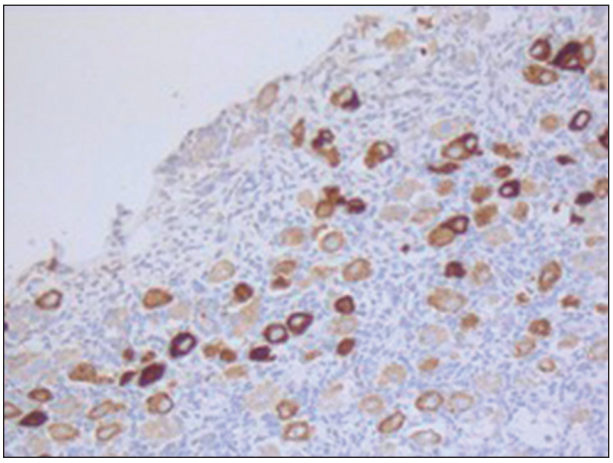

Figure 6. Ovary of a 34-week-old fetus. Marked differences regarding the degree of immunoreactivity for Oct-4 among adjacent follicles. Immunostaining is restricted to the cytoplasm of oocytes, ranging from strong to weak; 10x.

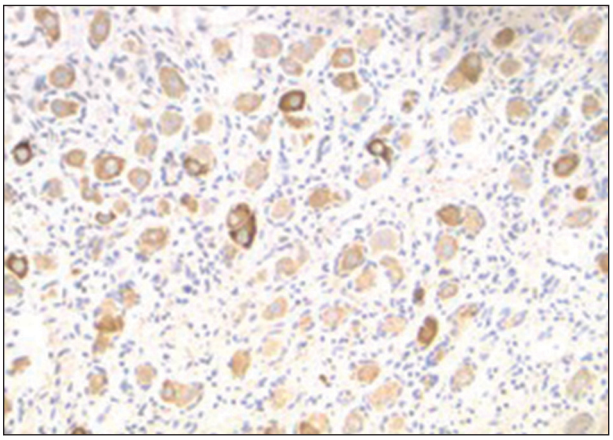

Figure 7. Ovary of a 38-week-old fetus. Primordial follicles are evidenced by strong immunostaining for Oct-4, mainly localized in the cytoplasm of oocytes. Primary follicles show mild expression of Oct-4; 10x.

Discussion

Our data show that Oct-4 is highly expressed during development in human ovaries. Reactivity for Oct-4 was observed at all gestational ages analyzed, from 12 to 38 weeks of gestation. These

findings confirm a major role for Oct-4 in human ovary development.

Reactivity for Oct-4 changed from one case to the next, according to the different stages of development, indicating major changes in Oct-4 expression during gestation. In the early stages of ovarian development, immunostaining for Oct-4 allowed the identification, in the outer cortex, of small groups of ovarian progenitors, probably representing the ovarian stem cell niches. These findings indicate Oct-4 as a useful marker for the identification of ovarian stem cell niches in the fetal human ovaries.

Regarding the cell types immunostained by Oct-4, our data show that Oct-4 is expressed in different cell types in the developing human ovaries: i) large cells (oogonia); ii) small-sized pluripotent cells; iii) very small embryonic-like stem cells; iv) oocytes of primordial follicles. Moreover, Oct-4 was expressed in different cell compartments in these cell types, being mainly localized at nuclear level in stem/precursor cells, and restricted to the cytoplasm in oocytes. The significance of this change in subcellular distribution, at the best of our knowledge, is not well understood.

Changes in Oct-4 expression were observed in oocytes according to the different gestational age, characterized by a progressive decrease in immunoreactivity during gestation, ending with a low Oct-4 expression at birth. Primordial follicles were characterized, in all cases, by higher levels of expression of Oct-4, when compared with primary follicles.

Regarding the previously reported expression of Oct-4 in granulosa cells [12], this finding was not confirmed by our findings. No significant reactivity for Oct-4 was detected, in this study, in follicular cells. As a consequence, the hypothesis that these cells might maintain stemness and a transdifferentiation potential may not be confirmed [12]. In conclusion, the strong expression of Oct-4 here described in ovarian stem/progenitor cells, particularly in the initial phases of development, suggests a major role for Oct-4 in the development of human ovaries. Moreover, the strong cytoplasmic expression in oocytes indicates a role for Oct-4 in folliculogenesis. The predominant nuclear expression in progenitor cells and the cytoplasmic restriction of Oct-4 in oocytes suggest a different significance of nuclear and cytoplasmic reactivity that deserves further studies. The finding of a high expression of Oct-4 in primordial follicles might allow a new interpretation of the molecular mechanisms involved in human folliculogenesis and oogenesis, with possible implications on new therapeutic approaches in clinical practice for premature ovarian failure and infertility [17].

Declaration of interest

The Authors declare that there is no conflict of interest.

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