Research Article

Immunoexpression of Stat٣ in Human Fetal Ovary During the Second Trimester Pregnancy.

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Abstract
Background: In the development of the human fetal ovary, the second trimester includes the transition from oogonial replication to primordial follicle formation. The STAT family proteins plays an important role during germ cells differentiation in the human fetal ovary. The study was conducted at Warwick medical school research institute, UK. to assess differentiation of germ cells in a series of female gonads from the second trimester using STAT٣ immunoflorescence technique. **Materials and Methods:** Human fetal ovaries were obtained from second trimester fetuses (١٧ to ٢٤ weeks gestation) after induced abortion. Then the tissues were cut into Pieces for processing for paraffin sections. Ovarian pieces were studied for the presence of germ cell marker using immunohistochemistry for STAT٣. **Results:** STAT٣ was expressed in the cytoplasm of oocytes during oogenesis and folliculogenesis in human fetal ovary. While there was no expression in somatic cells. When STAT٣ was activated, it moved from the cytoplasm to the nucleus. **Conclusions:** STAT٣ is a useful tool for germ cell studies; the expression of STAT٣ in human fetal ovary enable one to follow oogenesis throughout the second trimester pregnancy. So, the present study will serve as a reference for further analyses investigating the developing human fetal ovary.

Key words: STAT٣, Human fetal ovary, Second trimester.

Introduction
STAT٣ is one of a series of cytosolic STAT (signal transducer and activator of transcription) proteins known to be involved in transcriptional regulation.(١) STAT proteins are activated by hormones, growth factors and extracellular signaling proteins such as cyto-kines.(٢) The STAT members are seven proteins (STAT ١, ٢, ٣, ٤, ٥A, ٥B, and ٦) which are identified in mammals and all are important for cellular signaling.(٣) Activated STAT٣ is required for dimerization, nuclear translocation and transcriptional activity.(٤)

STAT٣ has a critical role in different biological functions such as cell growth, survival and motility depending on the stimulus and the cell type.(٥) It is essential during embryogenesis and its disruption is embryonic lethal.(٦) Also, during implantation and decidualization, it is important for mouse and rat reproduction.(٧) Additionally, leptin regulates ovarian granulose cell function through JAK/STAT pathway. STAT٣ pathway is involved in mediating epidermal growth factor (EGF) which stimulates ovarian follicular development.(٨)

STAT٣ protein is expressed in the cytoplasm of oocytes from primordial, primary and secondary follicles in the adult ovary and in developing acrosomes of round spermatids in the adult testis. STAT٣ is also expressed in gonocytes, prospermatogonia and oocytes of embryonic and neonatal gonads.(٩)

The present study was carried out to assess differentiation of germ cells in a series of female gonads from the second trimester. The oocyte marker STAT٣, has been used because it played an essential role in gem cell different-tiation in mice.

Materials and Methods
Tissue samples:
The present study was approved by Warwickshire Research Ethics Committee UK.

Human fetal ovaries were obtained from second trimester fetuses (١٧ to ٢٤ weeks gestation) after induced abortion. None of the terminations were for reasons of fetal abnormality. Gestational age was estimated through ultrasound examination before termination. Ovaries were dissected carefully from the fetal abdominal cavity and placed in collection medium for preservation.[١٠]

The human fetal ovaries were cut into uniform sized wedges (٣x ٣x٣ mm). Freezing and thawing of ovarian tissue was done according.[١١] The ovarian fragments were transferred into cryovials (one fragment per vial) containing ١ml of a cryopreservation medium. The slow freezing procedure was performed in a programmable controlled rate freezing machine for two hours then all the vials were plunged into liquid nitrogen tank at -١٩٦°C.

Pieces of human fetal ovaries were processed for paraffin sections.[١٢]. To assure satisfactory quality, tissue samples were assessed by hematoxy-lineos in staining. Any poor sample was excluded from further analysis.

Fluorescent immunocytochemical staining
Human fetal ovarian pieces were studied for the presence of germ cell marker using immunefluoresence for STAT٣. The primary and secondary antibodies used are indicated in Table (I). Negative control was performed by omitting the primary antibody which was replaced by normal serum resulting in complete absence of signals and no staining was observed in this control.

Table (١): The primary and secondary antibodies for STAT٣.

Primary antibody	Company	Pre-treatment	Dilution	Secondary antibody	Dilution	Company
STAT٣ (C٢٠) Rabbit anti-human	Santa cruz, UK	Enzymatic	١:٥٠٠	Goat anti-rabbit IgG FITC	١:١٠٠٠	Sigma, UK

Statistical analysis
Data were presented as mean±standard deviation (SD). Statistical significance was determined by unpaired student's t-Test of unequal variance. The relashionship between age (gestation weeks) and the number of positive STAT٣ stained cells was investigated using pearson correlation coefficient. Results were considered significant at $p<٠.٠٥$.

Results

From ١٧ weeks to ٢٤ weeks the occurrence of STAT٣ immunofluorescence in human oocytes was detected by the use of high power view of a scanning laser confocal microscopy. Negative controls were shown in (Figure ١).

STAT٣ was not detected at ١٨ weeks gestation but began to be expressed at ١٩ weeks gestation in developing human ovary.

STAT٣ protein immune-fluorescence was seen in the cytoplasm of all oocytes within oocytes nests (Figure ٢), in early follicles and in primary follicles at the interface with the follicle cells (Figure ٣). Oocytes in early follicles after nest breakdown were clearly stained for STAT٣ in the cytoplasm, but there was no labelling of somatic stromal or granulosa cells with STAT٣ (Figure ٣, ٤ and ٥). Cytoplasm was stained for STAT٣ at similar intensity for small and growing oocytes (Figure ٢, ٣ and ٥). STAT٣ became progressively stronger in the cytoplasm of oocytes as age advanced while there was no expression in somatic cells.

The stained sections confirmed that STAT٣ is a germ cell (oocyte) marker in humans (Figure ٥). When STAT٣ is activated, it moves from the cytoplasm to the nucleus. At ٢٤ weeks gestation, there was a strong nuclear staining (Figure ٤).

Figure ٦ summarizes the results of a quantitative assessment of the STAT٣ marker at different gestational ages (weeks).
There was a strong positive correlation between the age (gestation weeks) and the number of STAT٣ positive stained cells (Table ٢).
There was a statistically significant differ-ence at the P ٠.٠٥ level in the number of STAT٣

positive stained cells in relation to age between the eight groups (P=٠.٠٠٣) (Table ٣).

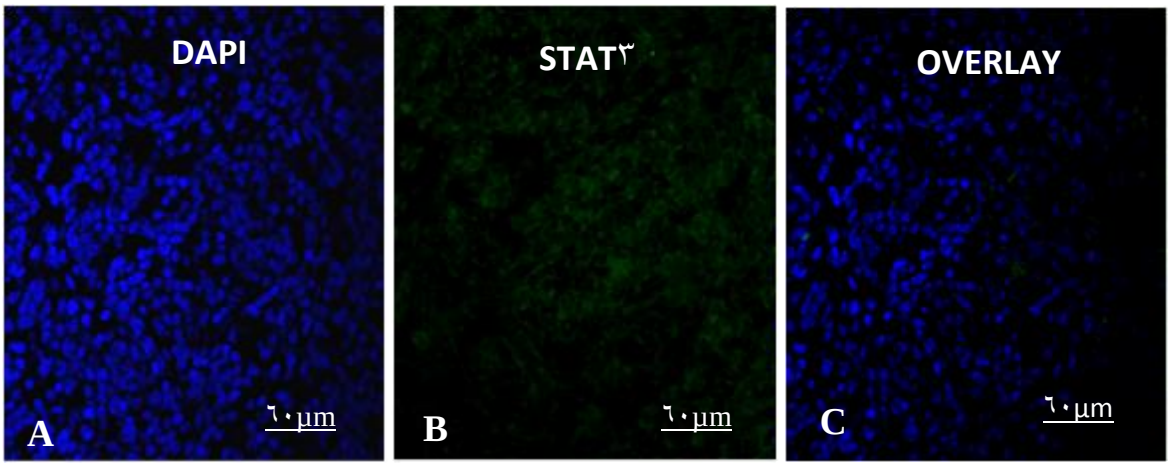

Figure (١): Photomicrographs of a section in fetal ovary (gestational age ١٩ weeks) showing negative control for STAT٣. (A) Shows confocal immunostained section with DAPI as a nuclear stain, (B) Shows no signal in absence of primary antibody for STAT٣ and (C) Shows overlay.

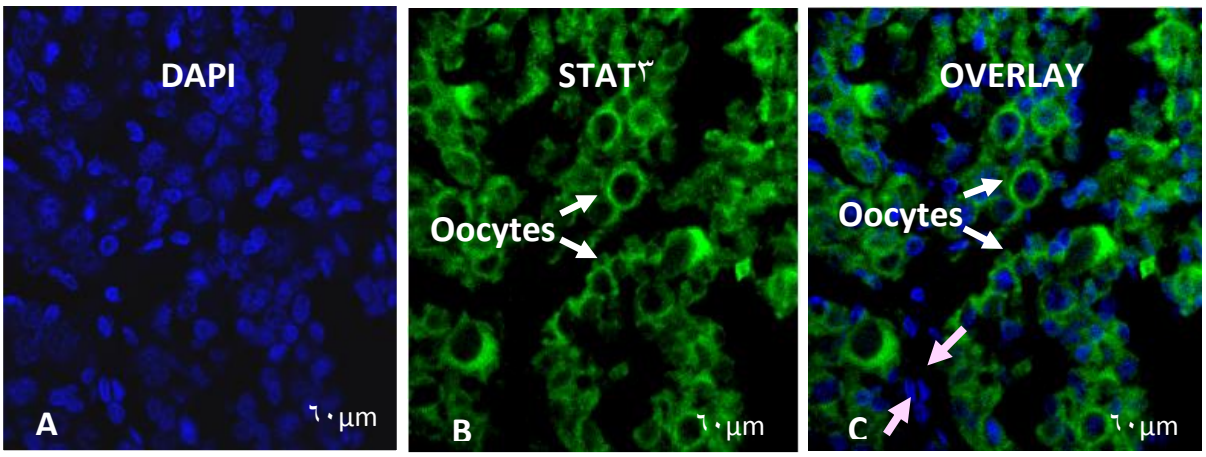

Figure (٢): Photomicrographs of a section in fetal ovary (gestational age ٢١ weeks). Arrows in (B) and (C) indicate oocytes with cytoplasmic labeling. Arrows in (C) indicate unstained somatic cells.

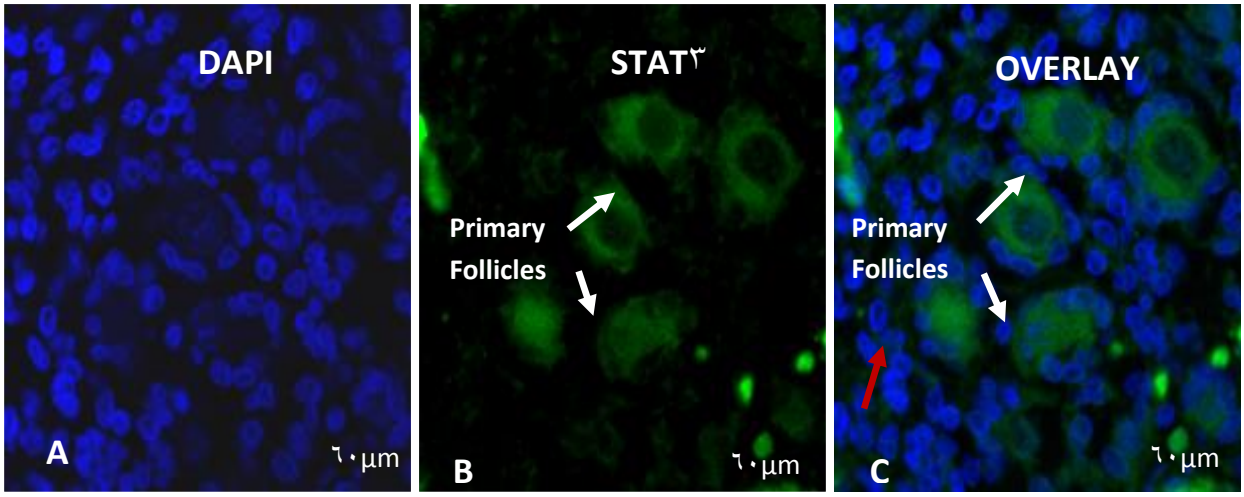

Figure ٣: Photomicrographs of a section in fetal ovary (gestational age ٢٣ weeks). Arrows in (B) and (C) indicate cytoplasmic labeling of oocytes enclosed in follicle cells, red arrow indicates absence of labeling in granulosa cells

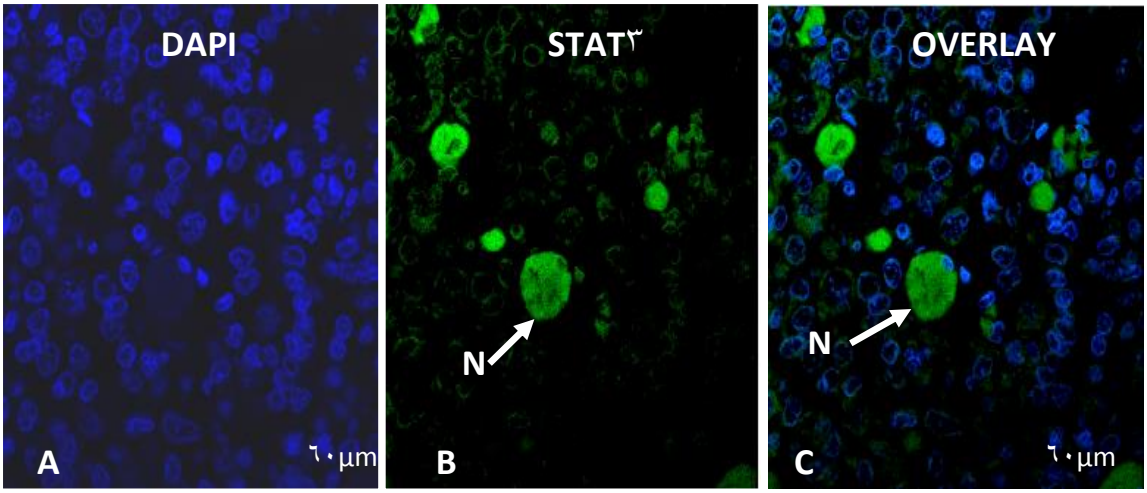

Figure ٤: Photomicrographs of a section in fetal ovary (gestational age ٢٤ weeks). Arrows in (B) and (C) indicate strong nuclear labeling after STAT٣ protein activation and movement from oocyte cytoplasm to oocyte nucleus.

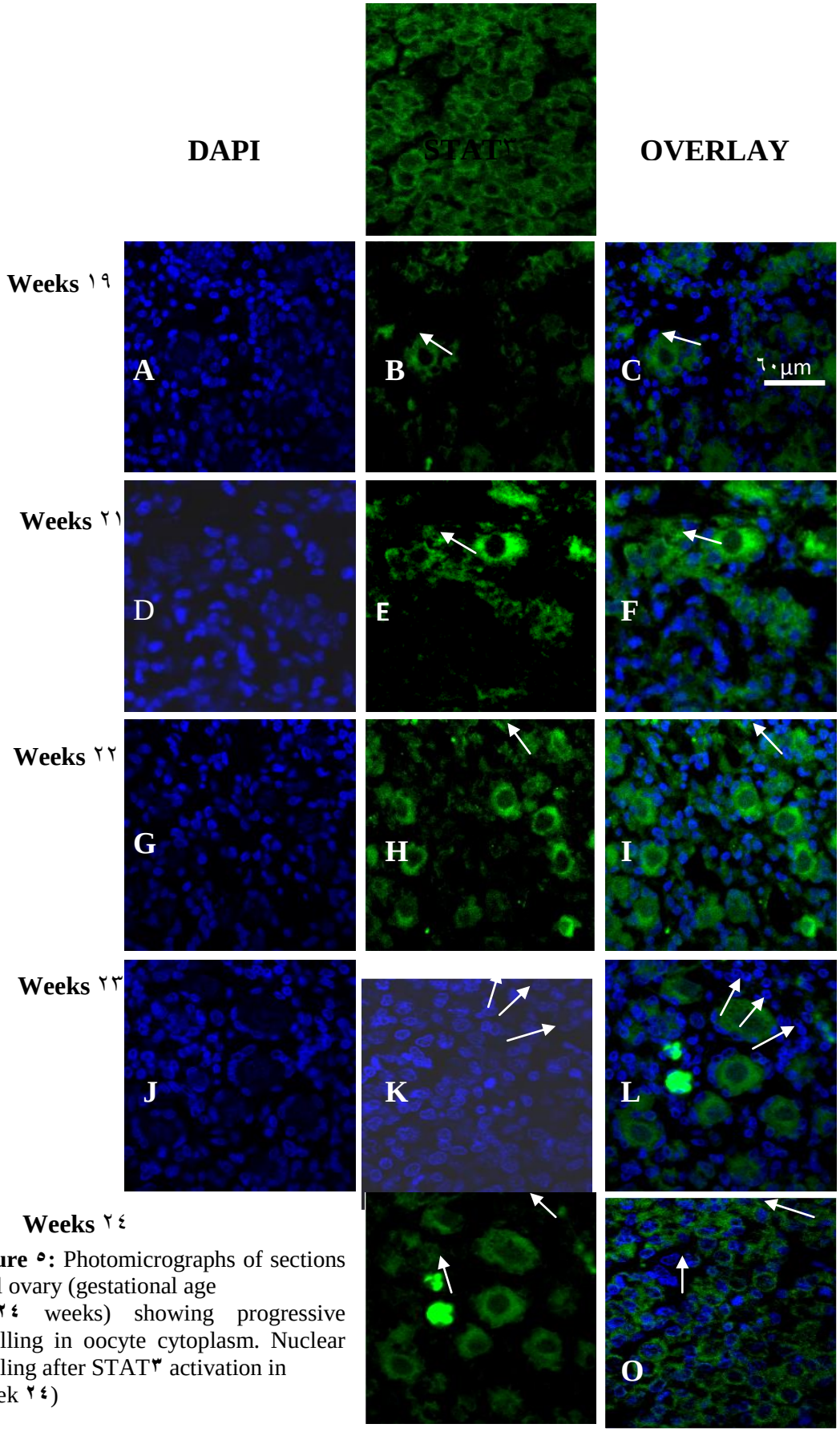

Figure ٥: Photomicrographs of sections in fetal ovary (gestational age ١٩-٢٤ weeks) showing progressive labelling in oocyte cytoplasm. Nuclear labeling after STAT٣ activation in (week ٢٤)

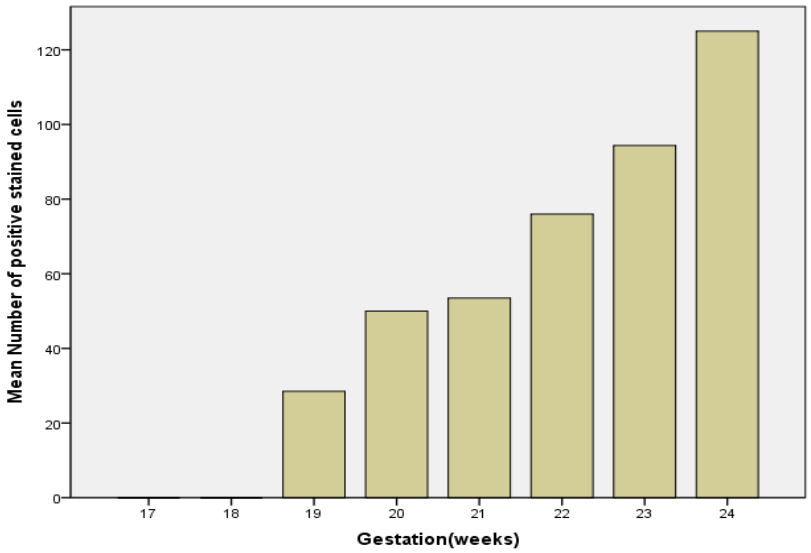

Figure ٦: The mean number of STAT٣ positive stained cells at different gestational ages (weeks).

Table ٢: The relationship between age (gestation weeks) and the number of STAT٣ positive stained cells using pearson correlation coefficient.

		Gestation(weeks)	Number of positive stained cells
Gestation(weeks)	Pearson Correlation	١	.٨٩٣**
	Sig. (٢-tailed)		.٠٠٠
	N	٤٩	٤٩
Number of positive stained cells	Pearson Correlation	.٨٩٣**	١
	Sig. (٢-tailed)	.٠٠٠	
	N	٤٩	٤٩

Table ٣: A one way analysis of variance (ANOVA) between age groups to explore the impact of age on the number of STAT٣ positive stained cells. Significance level ($p \leq ٠.٠٥$ level).

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	٢٢٠٧١.٧٥٧	٧	٣١٥٣.١٠٨	٦.٩٦٩	.٠٠٣
Within Groups	٤٩٧٦.٨٧٥	١١	٤٥٢.٤٤٣		
Total	٢٧٠٤٨.٦٣٢	١٨			

Discussion

In the present study, human oocytes showed STAT٣ protein immunofluorescence in the cytoplasm of all oocytes within oocytes nests, early follicles and primary follicles at the interface with the follicle cells, oocytes in early follicles after nest breakdown were clearly stained for STAT٣ in the cytoplasm. Cytoplasm was stained for STAT٣ at similar intensity for small and growing oocytes. This is in agreement with Wen et al. (٢٠٠٦).[٨] who demonstrated that STAT٣ is expressed in high levels in ovary.

In agreement with Antczak and Blerkom [١٣] and Murphy et al.[١٥], there was no labelling of somatic stromal or granulose cells with STAT٣. Explanation of this notion is that STAT٣ interact with oocyte cytoplasmic receptor like molecule to involve a signal transduction pathway that is somewhat different from the Jak-STAT pathway in somatic cells.[١]

STAT٣ was not detected at ١٨ weeks gestation but began to be expressed at ١٩ weeks gestation in developing human ovary. Murphy et al.[٩]; Hou et al.[١٤] reported that the immunoexpression of STAT٣ was not detected at ١٢.٥ day post coital (dpc) but began at ١٣.٥ (dpc) in developing ovaries of mice. These periods are equivalent to the second trimester pregnancy in human.

In the present study, in the developing ovary, STAT٣ became progressively stronger in the cytoplasm of oocytes while there was no expression in somatic cells. This is supported by the findings of Murphy et al. and Hou et al. in mice[٩, ١٤].

When STAT٣ is activated, it moves from the cytoplasm to the nucleus. At ٢٤ weeks gestation, there was a strong nuclear staining. Therefore, STAT٣ was activated in some germ cells. Up to the researcher knowledge, this finding was not supported by previous research. Murphy et al.[٩] did not observe nuclear staining at any examined mice ovary. Therefore, STAT٣ was not active in mice germ cells.

Antczak and Blerkom [١٣] explained the process of STAT٣ activation; Cytoplasmic STAT٣ proteins are phosphorylated by a Jak tyrosine kinase following the activation of a particular set of receptors. Phosphorylated STAT proteins form homo- or heterodimers and rapidly translocate to the nucleus to initiate specific gene transcription. Also, Matsuoka et al.[١٥] results suggest possible roles of leptin in several aspects during oocyte maturation by activating the STAT signal transduction pathway.

In the present study, there was a strong positive correlation between the gestational age and the numbers of STAT٣ positive cells. This finding is in agreement with Murphy et al.[٩]

It could be concluded that STAT٣ is a useful tool for germ cell studies; the expression of STAT٣ in human fetal ovary enable one to follow oogenesis throughout the second trimester pregnancy. So, the present study will serve as a reference for further analyses investigating the develop-ping human fetal ovary.

Since, human fertility depends upon a supply of oocytes (eggs) being available for ovulation during the adult life of the woman. The present study will add to other studies that aim to find out the factors that control the ovarian reserve of oocytes that last throughout the female reproductive period. This might help in solving female infertility by finding novel ways that ensure sound oocyte supply to be fertilized.

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