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# Cellular functions of spermatogonial stem cells in relation to JAK/STAT signaling pathway

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This manuscript comprehensively reviews the interrelationship between spermatogonial stem cells (SSCs) and the JAK/STAT signaling pathway. Spermatogonial stem cells in the testes of male mammals, characterized by their self-renewal and pluripotential differentiation capabilities, are essential for tissue regeneration, immunomodulation, and advancements in regenerative medicine. This review delves into the historical background and biological characteristics of SSCs, with a particular emphasis on the pivotal role of the JAK/STAT signaling pathway in their proliferation, maturation, and differentiation processes. Research indicates that the JAK/STAT pathway extensively influences various functionalities of spermatogonial stem cells, encompassing immunomodulation, tissue differentiation, homing, and adaptation to the microenvironment. Herein, we collate and dissect related studies, shedding light on the intricate dynamics between SSCs and the JAK/STAT signaling pathway, and examine the implications of these interactions on the biological attributes and functionalities of SSCs. Furthermore, the review discusses the profound implications of these findings for preclinical research and the domain of cellular engineering. It is acknowledged that, despite advancements in the research of SSCs and the JAK/STAT signaling pathway, investigations in humans and larger mammals remain inadequate, necessitating more in-depth exploration to establish a comprehensive theoretical framework. Overall, this review offers an invaluable reference for deciphering the mechanisms of the spermatogonial stem cell signaling pathways and establishes a theoretical groundwork for related preclinical research.

KEYWORDS

**spermatogonial stem cells, JAK/STAT signaling pathway, cellular functions, immune, differentiation**

## Introduction

Spermatogonial stem cells, a distinct cellular entity within the testes of male mammals, demonstrate extraordinary self-renewal and pluripotential differentiation capabilities (Lin et al., 2022; Qin et al., 2023). These cells are not only pivotal in tissue regeneration and immunomodulation but are also increasingly recognized for their prospective contributions to regenerative medicine, emerging as a novel source for stem cell therapies (Hashemi Karoii and Azizi, 2023). Recent research has evolved from focusing on the *in vivo* colonization, survival, and differentiation of spermatogonial stem cells post-transplantation to examining their interactions with the microenvironment and

immunomodulatory roles (Hashemi Karoii and Azizi, 2023). Specifically, the regulatory dynamics between spermatogonial stem cells and their target cells within signaling pathways have garnered substantial research interest. In the realm of tissue engineering, spermatogonial stem cells are integral to tissue repair and organogenesis. Their interactions with cellular signaling pathways elucidate their functional mechanisms. Notably, the JAK-STAT (Janus kinase signal transducer and activator of transcription) signaling pathway, which is instrumental in vital cellular processes such as proliferation, maturation, and differentiation, exerts a profound influence on the functions of spermatogonial stem cells and their target cells (Shields et al., 2014; Zhang et al., 2015). This review aims to summarize the advancements in research on the JAK/STAT signaling pathway and the multifaceted cellular functions of spermatogonial stem cells, encompassing immunoregulation, tissue differentiation, homing, and microenvironmental adaptation. The findings discussed herein are intended to offer a critical reference for elucidating the signaling pathway mechanisms underlying functional changes in spermatogonial stem cells and to provide theoretical underpinnings for related preclinical studies and cellular engineering research.

## Spermatogenic stem cells

The term "stem cell" was first coined in 1901 by Regaud, who, in his study of spermatogonia in the testes, postulated the existence of a stem cell system to replenish the plethora of differentiated cells (Regaud, 1901). Presently, it is acknowledged that multiple stem cell types exist, with adult stem cells being a prominent category. An adult stem cell, located in specific tissues, possesses the dual capacity to both proliferate, generating new stem cells, and differentiate, yielding more progenitor cells (Dym et al., 2009). Spermatogonial stem cells, a subset of adult stem cells from the testes, have the unique ability to self-renew, sustaining their population, and differentiate, giving rise to a multitude of daughter cells (de Rooij and Russell, 2000). These spermatogonia are situated at the basement membrane of the seminiferous tubules and initially exist as single cells (As). Upon division, these cells can either independently form two as spermatogonia or, by connecting through cell bridges, form two pairs of spermatogonia (Apr). Subsequently, Apr spermatogonia divide to yield pairs (Apr), which then divide to form chains of 4, 8, or 16 cells (Aal). Following approximately 6-7 mitotic divisions, these cells develop into spermatocytes, progressively migrating away from the basement membrane towards the tubular lumen, undergoing meiosis to transform into spermatids, and ultimately entering the tubular lumen. Collectively, As, Apr, and Aal spermatogonia are categorized as undifferentiated spermatogonia. Given the lack of 100% purified SSCs, the SSCs under investigation are also referred to as undifferentiated spermatogonial stem cells (de Rooij and Russell, 2000). The existence of SSCs has been acknowledged since the 1950s, although research progress was initially sluggish (Nakagawa et al., 2007). A primary reason for this was the reliance on traditional research methodologies that focused predominantly on the morphology of SSCs, rather than exploring molecular mechanisms (de Rooij and Russell, 2000). However, around 1990-2000, there was a significant paradigm shift, marking the onset of a new era in SSC research. This change was catalyzed by two pivotal technological breakthroughs: the first being the development of the testis transplantation technique by Brinster and Avarbock (1994), which spurred unprecedented advancements in SSC research (Brinster and Avarbock, 1994; Brinster and Zimmermann, 1994). The second milestone was achieved in 2003 when Kanatsu-Shinohara and his team established a protocol for the long-term *in vitro* culture and proliferation of SSCs (Kanatsu-Shinohara et al., 2003; Kanatsu-Shinohara et al., 2005; Kubota and Brinster, 2006). These methodologies have enabled the transformation of SSCs into pluripotent stem cells and their genetic manipulation *in vitro*. Recent studies report that pluripotent stem cells derived from neonatal mice, adult mice, and human testicular tissue can, *in vitro*, differentiate into various cell lines capable of forming teratomas upon injection into immunodeficient mice (Kanatsu-Shinohara et al., 2004; Guan et al., 2006; Conrad et al., 2008). Additionally, gene targeting and homologous recombination techniques in SSCs have led to the successful creation of knockout mice, and while knockout rats have not yet been produced, successful homologous recombination in the rat genome has been achieved (Kanatsu-Shinohara et al., 2006; Kanatsu-Shinohara et al., 2011; He et al., 2015). For the cell culture aspect of SSCs, there are quite a few experiences to learn. SSCs were isolated from the testes of C57BL/6J mice or SD rats on the 7th day after birth. Subsequently, after removal of the tunica albuginea and epididymal curvature, the seminiferous tubules were excised and flushed with Hanks balanced salt solution (HBSS). They were physically sheared and digested with a solution of DnaseI, hyaluronidase, collagenase, and trypsin using a two-step enzymatic digestion method in which the digestive enzymes included DnaseI, hyaluronidase, collagenase, and trypsin. The dissociated single cell suspension was resuspended and cultured.

Spermatogonial stem cells also exhibit several distinctive biological properties. SSCs are characterized by their inherent potential for self-replication and differentiation. De Rooij (1988) identified as cells as the spermatogenic stem cells in various animals, including rats, mice, Chinese hamsters, and sheep. Dym (1994) categorized undifferentiated spermatogonia into five subpopulations (A0—A4) based on morphological characteristics. A0 cells divide slowly, are in a dormant state, and can reintegrate into the spermatogonial lineage, while A1—A4 cells are proliferative, with A4 cells being true stem cells that not only generate differentiated In and B cells but also new A2 cells, thus maintaining the stem cell count within their population. Under conditions such as X-ray irradiation or treatment with the alkylating agent leucovorin, as cells exhibit a degree of damage tolerance, whereas Apr and subsequent spermatogonial stages succumb or are depleted. Upon removal of the damaging conditions, the surviving As cells can regenerate the entire spermatogenic cell lineage through self-replication and differentiation (Huckins, 1971). SSCs possess a unique trait of immortality. While other adult tissue stem cells are capable of self-renewal, they do not contribute genetic information to offspring, thus not influencing the genetic makeup of the progeny. In

contrast, oocytes transmit genetic information but lose self-replicative ability post-birth (Brinster and Zimmermann, 1994). SSCs uniquely embody both aforementioned capabilities, making them the only replicating population of diploid cells in the adult body, hence considered immortal (Brinster and Avarbock, 1994) (Figure 1) (Images in this article were generated by Midjourney).

## JAK/STAT pathway

Janus Kinase (JAK) is a family of non-receptor tyrosine protein kinases comprising four members: Janus Kinase 1, Janus Kinase 2, Janus Kinase 3, and Tyrosine Kinase 2. Cytokine activation triggers the opening of the intracellular structural domain of the receptor, facilitating its binding to JAK. This interaction leads to the phosphorylation of JAK, forming p-JAK, which in turn recruits intracellular STAT transcription factors. The phosphorylated STAT transcription factors then dimerize and polymerize, enabling them to recognize and bind to specific regions of the DNA, thus modulating gene expression within the target cell. This cascade of events delineates the JAK/STAT signaling pathway (Stark and Darnell, 2012).

The JAK/STAT pathway is a common conduit utilized by many cytokines, capable of transducing diverse extracellular signals into specific cellular responses (Cao et al., 2015; Amano et al., 2016; Ahmad et al., 2017; Browning et al., 2018). STAT molecules, central to this pathway, can form various structures including homodimers, heterodimers, or tetramers, and subsequently translocate to the nucleus or mitochondria to bind DNA and regulate the transcription of target proteins (Brinster and Zimmermann, 1994; Wei et al., 2010; Begitt et al., 2014; Meier and Larner, 2014; Villarino et al., 2015). Moreover, the JAK/STAT pathway is subject to negative feedback regulation, crucial for maintaining intracellular homeostasis. Post-activation, suppressors of cytokine signaling (SOCS) are released, inhibiting JAK phosphorylation and thereby modulating the expression of target proteins (Brinster and Zimmermann, 1994; Wei et al., 2010; Begitt et al., 2014; Meier and Larner, 2014; Villarino et al., 2015). This negative regulation, exemplified by SOCS, plays a vital role in cellular equilibrium (Tamiya et al., 2011).

The influence of the JAK/STAT pathway on gene expression is both specific and extensive (Stark and Darnell, 2012; Villarino et al., 2015). Regarding specificity, different cytokines may activate identical STAT molecules, such as interleukin 6, interleukin 27, and interleukin 10 activating STAT3, yet their effects on cellular functions vary. For instance, interleukin 10 exerts a pain-inhibitory effect and promotes neural repair via STAT3 activation, whereas interleukin 6, through the same pathway, impedes neural repair and induces nociceptive sensitization (Meka et al., 2015; Zhu et al., 2017; Uciechowski and Dempke, 2020). This specificity stems from varying mechanisms within the signaling pathways, where distinct cytokine stimuli lead to diverse cellular responses through the activation of other signaling cascades, ultimately affecting gene transcription and translation, and resulting in specific cellular functions.

In terms of broader impact, STAT molecules can bind to multiple sites on the DNA, influencing gene expression by attaching to promoter or enhancer regions. For example, interleukin 4 activates STAT6 molecules in T cells, which then bind to enhancer regions in T cell nuclei, prompting phenotypic changes and differentiation into helper T 2 cells (Xu et al., 1996; Kanno et al., 2012; Vahedi et al., 2012) (Figure 2).

## Relationship between spermatogonial stem cells and JAK/STAT pathway

Recent developments have led scholars to transition from perceiving spermatogonial stem cells (SSCs) as monopotential to recognizing them as multipotential stem cells. In 2004, Kanatsu-Shinohara et al. (2004) first cultured multipotential embryonic-like stem cells from neonatal mouse SSCs and successfully induced their transdifferentiation into various adult cell types. A parallel study by Guan et al. (2006), akin to that of Kanatsu-Shinohara et al. (Conrad et al., 2008), yielded similar results, producing multipotent adult male germline stem cells (maGSCs) derived from adult mouse testis. In 2007, GPR125 positive germline progenitor cells from adult mice were isolated and cultured, yielding embryonic-like stem cells that were further transdifferentiated into cells of other embryonic lineages, even contributing to chimeric formations (Seandel et al., 2007). Both induction systems generated multipotent embryonic-like stem cells, yet the origin of these cells and the mechanisms underlying their dedifferentiation remained elusive.

In 2010, to gain insight into the dedifferentiation process of SSCs, researchers delineated it into three phases: the SSC stage, intermediate-type SSCs (iSSCs), and the embryonic-like stem cell stage (Kim et al., 2010). Takashima et al. (2013) suggested that the autonomous reprogramming of SSCs might be influenced by unstable DNA methylation and the Dmrt1-Sox2 cascade reaction, critical in regulating SSC multipotency. Spermatogonial stem cells have been shown to differentiate into specific cell types suitable for transplantation *in vitro*, such as vascular endothelial and smooth muscle cells (Im et al., 2012), renal parenchymal cells (Wu et al., 2008; Heer et al., 2013; De Chiara et al., 2014), omphalocele-like cells (Wang et al., 2012), neuroepithelial-like cells (Nazm Bojnordi et al., 2013), neuronal-like cells (Liu et al., 2012), and hepatic stem cell-like cells (Zhang et al., 2013). However, studies have consistently reported that transplanted SSCs exhibit low *in vivo* colonization rates and brief survival times, implying alternative mechanisms of action.

The interaction between the JAK/STAT signaling pathway and spermatogonial stem cells is twofold: ① The JAK/STAT pathway in target cells modulates the biological properties of SSCs, exemplified by support cells signaling to SSCs via the JAK-STAT pathway, thereby shaping the microenvironment conducive to SSC self-renewal (Tulina and Matunis, 2001). ② The JAK/STAT pathway can influence the biological attributes of SSCs themselves; for instance, Brawley and Matunis (2004) demonstrated that mitotically active spermatogonia could repopulate their niche and revert to a stem cell state when JAK-STAT signaling was conditionally altered (Brawley and Matunis, 2004). Therefore, the exploration of the relationship between the biological

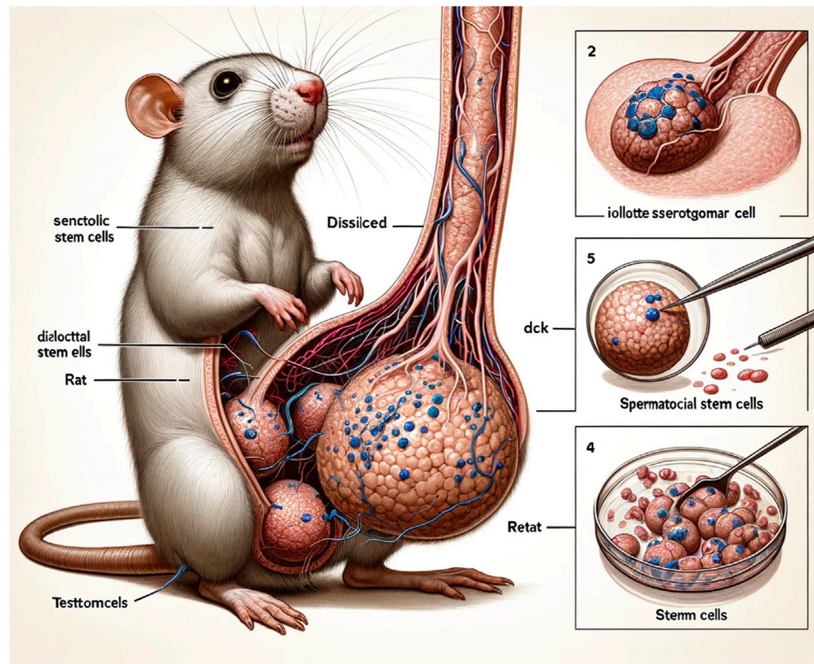


FIGURE 1
Spermatogonial stem cells, isolated, purified and cultured from rat testes.

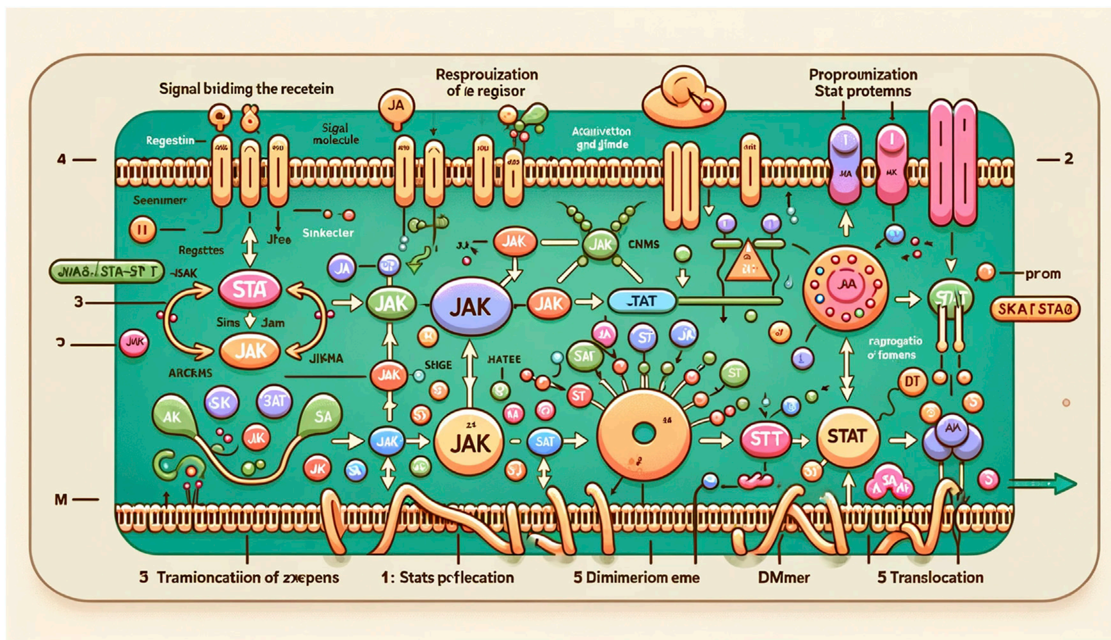


FIGURE 2
Diagram of the JAK-STAT signaling pathway: 1) Signal molecule binding to the receptor, 2) Activation of JAK kinase and phosphorylation of the receptor, 3) Recruitment and phosphorylation of STAT proteins by JAK, 4) Dimerization of STAT proteins, 5) Translocation of STAT dimers into the nucleus and initiation of gene transcription.

characteristics of SSCs and the JAK/STAT pathway can be approached from two perspectives: firstly, examining the impact of the target cell's JAK/STAT pathway on SSCs (Tulina and Matunis, 2001); and secondly, investigating the influence of the SSC's own JAK/STAT pathway on its biological properties (Brawley and Matunis, 2004).

## Regulation of spermatogonial stem cells by the target cell JAK/STAT pathway

The influence of the JAK/STAT pathway in target cells on spermatogonial stem cells (SSCs) primarily manifests in aspects such as stem cell self-renewal, dedifferentiation, proliferation, development, and the immune microenvironment. Sheng et al. (2009a) discovered that a subset of mesenchymal stromal cells, termed "hubs" in *Drosophila* testis, activate the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway in neighboring germ cells and somatic stem cells. This activation is crucial for maintaining germline stem cells (GSCs), creating a microenvironment favorable for GSC maintenance. Under certain conditions, spermatogonia can revert to GSCs when the JAK/STAT pathway is active. Conversely, when this pathway is inhibited, spermatogonia lose their dedifferentiation capability. STAT92E, a critical protein in the JAK/STAT pathway, is enriched in GSCs and progenitor cells' vesicles, with increased activation in spermatogonia near the testis apex, indicating local upregulation of JAK/STAT signaling during dedifferentiation. Unpaired (Upd), a ligand of the JAK/STAT pathway, does not vary in mRNA levels and distribution during dedifferentiation, suggesting its role in activating the pathway. The JAK/STAT pathway also contributes to cell motility, potentially related to cell rearrangements during spermatogonia's reversion to GSCs.

Wawersik et al. (2005) provided direct evidence of the JAK/STAT pathway mediating key signals from somatic gonads that regulate sexual development in the male germ line, highlighting its role in sex determination and development of SSCs. Brawley and Matunis (2004) found that the restoration of JAK/STAT signaling in the testis can regenerate GSCs, even from a complete absence. These regenerated GSCs were functional and could segregate from adjacent spermatogonia, reverting to stem cells. They demonstrated that spermatogonia, even after undergoing limited mitotic divisions, could reoccupy their niche and revert to stem cells under appropriate microenvironmental conditions. This finding implies that transit-amplifying cells can dedifferentiate into functional stem cells for tissue regeneration. Additionally, GSC differentiation occurs when the function of stat92E (*Drosophila* STAT homologue) is lost, but its restoration can revert differentiated spermatogonia back to stem cells. This process does not occur through symmetric division of remaining GSCs but through the restoration of already differentiated spermatogonia to stem cell identity, challenging previous theories on GSC regeneration.

Sheng et al. (2009b) showed that the JAK/STAT pathway activation in a subset of primordial germ cells (PGCs) linked to newly formed embryonic hubs leads to these PGCs expressing GSC markers and functioning as GSCs. In contrast, PGCs not linked to the hub began to differentiate, indicating the pathway's importance in establishing GSCs. Tulina and Matunis (2001) concluded that support cells activate the JAK-STAT pathway to signal neighboring stem cells, defining an ecological niche for stem cell self-renewal, emphasizing the pathway's critical role in maintaining SSC self-renewal and homeostasis.

In some studies, it has been shown (Morimoto et al., 2009; Marega et al., 2021) that Kit, a cell surface receptor in the JAK/STAT signaling pathway, and its downstream signaling pathway can activate STAT3, and the phosphorylated STAT forms a dimer and then enters the nucleus, which causes Kit to be significantly downregulated in certain phenotypes, thus affecting the behavior of cells. Some studies have shown (Morimoto et al., 2009) that in germline stem cells, Kit binding to SCF not only promotes cell survival and proliferation, but may also maintain their undifferentiated state by activating the JAK/STAT pathway. Although *in vivo* SSCs do not express the c-kit tyrosine kinase receptor (Kit), Kit expression is upregulated when cultured on laminin *in vitro*. Both Kit-positive and Kit-negative cells showed considerable levels of SSC activity after germ cell transplantation. Unlike differentiated spermatogonia, which depend on Kit for survival and proliferation, Kit on SSCs does not play any role in SSC self-renewal. Kit expression changes dynamically when SSCs begin to proliferate after germ cell transplantation. This implies that SSCs may upregulate Kit expression during active proliferation. Despite the limited role of Kit in SSC self-renewal, the regulation of Kit activation is crucial during subsequent differentiation. This may be related to the role of Kit in differentiated spermatogonia, which depend on Kit for survival and proliferation.

Hasan et al. (2015) demonstrated that the anti-apoptotic gene *Drosophila* inhibitor of apoptosis 1 (DIAP1), enriched in GSCs and CySCs and required for their survival, is a target of the JAK-STAT pathway. Enhanced JAK-STAT signaling upregulates DIAP1 in stem cells and their progeny, whereas decreased signaling increases their sensitivity to stress-induced apoptosis due to lower DIAP1 levels. Non-autonomous overexpression of DIAP1 in somatic cells rescued spermatogonia from stress-induced apoptosis, suggesting that microenvironmental signaling can promote stem cell survival by upregulating anti-apoptotic proteins, a potential general strategy for stem cell apoptosis resistance.

## Regulation of biological properties of spermatogonial stem cells by JAK/STAT signaling pathway

### The relationship between the JAK/STAT pathway and spermatogonial stem cell proliferation

The self-renewal and maintenance of spermatogonial stem cells (SSCs) have been the focus of extensive research (Kubota et al., 2004a; Kubota et al., 2004b; Oatley et al., 2006; Oatley et al., 2007; Oatley et al., 2009; Wu et al., 2010; Oatley et al., 2011). The JAK-STAT signaling pathway is pivotal for the maintenance of male SSCs in early male larvae and is also essential for inhibiting spermatogenic differentiation at this developmental stage. As hub formation occurs, this signaling center becomes progressively restricted to the anterior end of the embryonic gonad, promoting male SSC maintenance via differential cell adhesion and localized Jak-STAT pathway activation (Kubota et al., 2004a; Oatley et al., 2006; Oatley et al., 2007). The precise role of the Jak-STAT pathway in initiating SSC division, whether it actively regulates GSC-Hub adhesion formation or plays a more passive role by inhibiting PGC differentiation, is still under investigation (Kubota et al., 2004b; Oatley et al., 2009). These findings, however, underscore the importance of coordinated regulation of Jak-STAT signaling and central morphogenesis in

establishing functional SSCs (Kubota et al., 2004a; Kubota et al., 2004b; Oatley et al., 2006; Oatley et al., 2007; Oatley et al., 2009; Wu et al., 2010; Oatley et al., 2011).

In the *Drosophila* male germ line, SSC self-renewal heavily relies on JAK/STAT signaling (Kiger et al., 2001; Tulina and Matunis, 2001). Unlike invertebrates, mammals express five distinct STAT3 isoforms, with STAT3 identified as a critical regulator of pluripotency and self-renewal in mouse embryonic stem cells (Niwa et al., 1998; Raz et al., 1999). In *Drosophila*, the JAK-STAT pathway is triggered by a secreted ligand, UPD, whose binding to its receptor activates STAT and its translocation to the nucleus, regulating the transcription of STAT-responsive genes (Hombría and Brown, 2002; Arbouzova and Zeidler, 2006). Within the testis, UPD is expressed in hub cells, initiating the JAK-STAT signaling pathway in stem cells. Stem cells that maintain contact with the hub continue to receive signals for self-renewal, whereas those displaced from the hub experience reduced JAK-STAT pathway activation (Arbouzova and Zeidler, 2006). The intrinsic requirement of STAT for the maintenance of both SSCs and Cyst stem cells (CySCs) is evident, as depletion of STAT in testicular cells results in the loss of both stem cell populations, and individual SSCs or CySCs lacking STAT cannot be sustained. Conversely, when UPD is overexpressed in the testis apex, both stem cell populations self-renew independently of their proximity to the hub (Leatherman and Dinardo, 2008). Activation of STAT in somatic cells alone outside the ecotope leads to testes filled with ectopic CySCs and scattered self-renewing SSCs (de Cuevas and Matunis, 2011).

STAT activation in CySCs is both necessary and sufficient for maintaining somatic cells in the stem cell state (Leatherman and Dinardo, 2008). CySCs with pure mutations in STAT fail to self-renew and are rapidly lost from the testis apex. Interestingly, forced expression of structurally active JAK in somatic cyst cells leads to overproliferation of CySC-like cells. STAT-activated CySCs emit a signal promoting GSC self-renewal, potentially mediated by zinc finger homology domain protein 1 (Zfh1) (Leatherman and Dinardo, 2008). Zfh1, a target of STAT activation, is typically expressed only in CySCs and their immediate daughter cells. Ectopic expression of Zfh1 in cyst cells outside the niche mimics the phenotype of ectopic STAT activation: testes filled with ectopic CySCs and SSCs self-renewing away from the hub. Another target of activated STAT is chronologically inappropriate morphogenesis (Chinmo), which, when misexpressed in cyst cells, generates ectopic CySCs and SSCs (Flaherty et al., 2010). While zfh1 and chinmo are crucial for CySC self-renewal, their maintenance in SSCs does not directly require these proteins (Leatherman and Dinardo, 2008; Flaherty et al., 2010). Therefore, STAT may regulate stem cell maintenance through distinct downstream effectors in these two stem cell populations.

## Relationship between the JAK/STAT pathway and spermatogonial stem cell tissue differentiation

Normal spermatogenesis relies on the precise regulation of spermatogonial stem cell (SSC) differentiation, governed by intrinsic gene expression in stem cells and external signals, including soluble factors and adhesion molecules from the surrounding microenvironment (Donovan and Gearhart, 2001; Rey et al., 2001; Temple, 2001; Kubota et al., 2004b; Oatley et al., 2010; Kanatsu-Shinohara and Shinohara, 2013). Calcineurin, a unique family of single transmembrane structural domain glycoproteins, acts as key molecules during development, functioning as specific cell adhesion molecules in a Ca2+-dependent manner. Recent studies have highlighted the essential role of calmodulin-mediated cell adhesion in maintaining somatic stem cells in the *Drosophila* ovary and its involvement in signaling pathways (Son et al., 2002). Our previous research identified a novel adhesion molecule, short-type pb-calmodulin (STPB-C), in the testis, which plays a crucial role in promoting the survival of stem cell precursor gonadotrophs in neonatal rats (Wu et al., 2005). STPB-C activates the Janus kinase/signal transducer and transcriptional activation (JAK-STAT) signaling pathway by inducing the phosphorylation of JAK2 and STAT3, contributing to SSC differentiation (Wu et al., 2005).

The JAK-STAT pathway was initially identified as a key stimulator of SSC self-renewal and maintenance (Brawley and Matunis, 2004). STAT3, a member of the STAT family, is an important factor in regulating stem cell self-renewal and differentiation (Almiron Bonnin et al., 2018; Wei et al., 2018a; Wei et al., 2018b). In the testis, Stat3 is expressed in spermatogonia, and while its inhibition does not affect SSC proliferation and apoptosis, it impairs SSC differentiation, leading to an accumulation of undifferentiated SSCs (Oatley et al., 2010). The exact mechanism of Stat3's action is unclear, but Ngn3, a downstream target of STAT3 transcriptional activation, plays a significant role in controlling SSC and precursor spermatogonia differentiation (Kanatsu-Shinohara and Shinohara, 2013). Ngn3, predominantly expressed in undifferentiated spermatogonia, is regulated by Stat3, which binds to the promoter and enhancer regions of the Ngn3 gene. Deficiencies in Ngn3 exhibit spermatogenesis deficits similar to Stat3 deficiencies, suggesting a critical Stat3/Ngn3 pathway in SSC differentiation (Kaucher et al., 2012). Overexpression of Ngn3 promotes the expression of Stra8 and the SSC differentiation marker CD117, while suppressing the expression of the pro-proliferative factor PLZF, a key transcription factor in SSCs that balances self-renewal and differentiation through the JAK/STAT pathway (Costoya et al., 2004; Hobbs et al., 2010; Tang et al., 2014; Wu et al., 2014; Song et al., 2015; Liu et al., 2016; Mu et al., 2016). Removal of JAK-STAT signaling results in SSC differentiation into interconnected spermatogonial cysts expressing the differentiation marker Bag-of-Marbles (Bam), but when signaling is restored, spermatogonia near the hubs lose Bam expression, sever from cysts, and form single-functioning SSCs, demonstrating the plasticity of *Drosophila* spermatogonia and the ability of JAK-STAT signaling restoration to induce de-differentiation towards SSCs.

RNAs also significantly influence SSC differentiation (Moritoki et al., 2014; Zhou et al., 2016; Cheng et al., 2018; Mao et al., 2018; Zhang et al., 2020). miR-19b-3p positively alters Jak-Stat signaling through Plzf, enabling receptor dimerization, promoting Jak1 activation, and facilitating downstream Stat3 activation, thus regulating cellular self-renewal and differentiation of male germline stem cells in goats (Arbouzova and Zeidler, 2006). miR-135a, highly expressed in testes and SSCs, plays a crucial role in SSC maintenance by regulating the activity of forkhead box protein O1 (FoxO1). In cryptorchidism, the downregulation of miR-135a and aberrant FoxO1 activity reduce the number of SSCs transforming from spermatogonia to spermatocytes, indicating miR-135a′s essential role in spermatogenesis and SSC function (Zhou et al., 2016). miR-

135a is also involved in regulating the JAK/STAT pathway (Cheng et al., 2018; Mao et al., 2018), crucial for SSC development and germ cell proliferation and differentiation (Zhang et al., 2020), suggesting its potential impact on azoospermia via the JAK/STAT pathway. miR-34c activates the JAK2/STAT3 pathway, implicated in germ cell generation and SSC differentiation (Clotaire et al., 2018).

## The JAK/STAT pathway and immune regulation in spermatogonial stem cells

The pivotal role of the JAK/STAT signaling cascade in immune system development is evolutionarily conserved, with the specific details and cell types involved varying between *Drosophila* and mammals. Despite these differences, the focus on studying the immune functions of the JAK/STAT pathway remains consistent (Arbouzova and Zeidler, 2006). Regarding molecular function (Walker et al., 2009), miR-19b-3p regulates genes essential for transmembrane signaling receptor activity, chemokine activity, cytokine activity, chemokine receptor binding, and signaling receptor activity. Pathway annotation and enrichment analysis of the KEGG pathway indicate that miR-19b-3p mediates multiple pathways, including cytokine-cytokine receptor interactions and Jak-Stat signaling pathways, showing significant upregulation in both. Plzf is recognized as a key regulator in maintaining male germline stem cell (mGSC) self-renewal (Costoya et al., 2004; Song et al., 2013), contributing to testicular damage repair. However, Plzf also stimulates the development of myeloid tissues and plays a role in the immune response, both of which are essential for the development of the immune cell lineage, which is critical for defense against pathogens (Kovalovsky et al., 2008; Raberger et al., 2008; Savage et al., 2008; Dick and Sergei, 2009; Xu et al., 2009; Constantinides et al., 2014). One of the cytokines, Plzf, is localized in the nucleus and may interact with and attenuate the activity of phosphorylated Stat3, which is translocated in the nucleus, thereby regulating cellular self-renewal and differentiation of male germline stem cells in goats.

Stat3 transmits signals from cytokine and growth factor receptors in the plasma membrane to the nucleus, altering gene transcription. This transcriptional regulation involves Stat3 in key genes' transcriptional regulation, blocking apoptosis, promoting cell proliferation and survival, facilitating angiogenesis and metastasis, and suppressing tumor immune responses (Jing and Tweardy, 2005; Leeman et al., 2006; Frank, 2007; Germain and Frank, 2007; Regis et al., 2008; Johnston and Grandis, 2011; Shaim et al., 2018). Wei et al. (2021) explored the role of Doublesex and Mab-3 Related Transcription Factor 1 (Dmrt1) in balancing the intrinsic immune response of goat mGSCs, revealing a novel role of Dmrt1 in controlling reproductive immune diseases and showing that Dmrt1 regulates the inflammatory response in goat mGSCs by inhibiting the Toll-Like Receptor 4 (TLR4) signaling pathway. Dmrt1 also accelerates spermatogenesis and reduces apoptosis induced by the immune response (Niu et al., 2016; Wei et al., 2018b). TLR4, mainly localized in mGSCs, is responsible for the immune response in spermatogenic tubules, with Dmrt1 interacting with Plzf to reduce the immune response by inhibiting TLR4. These findings suggest Dmrt1 and Plzf as key factors in stimulating mGSC self-renewal and proliferation during the immune response.

Luu et al. (2014) demonstrated the interplay between the TLR and JAK/STAT signaling pathways, with STAT1 being directly recruited through TRAF6, indicating its crucial role in TLR-induced inflammation. Jiang et al. (2021) found that TLR4 is regulated by the JAK2-STAT3 pathway, with TLR4 activation indirectly initiating the JAK/STAT pathway. Cytokines released post-TLR4 activation, such as interferons or interleukins, can activate the JAK/STAT pathway. Hence, Dmrt1 and Plzf activate the JAK/STAT pathway through relevant pathways, implicating the JAK/STAT pathway in the immune response and inflammatory processes of spermatogonial stem cells.

## Relationship between the JAK/STAT signaling pathway and the microenvironment of spermatogonial stem cells

The microenvironment of spermatogonial stem cells (SSCs) is a complex and crucial factor influencing and maintaining the self-renewal state of SSCs. This definition encompasses structures and components surrounding SSCs, such as adjacent spermatogonia, SSCs themselves, supporting cells, peritubular myxoid cells, and various growth factors and cytokines bound to the extracellular matrix (de Rooij, 2009). The regulation of SSCs by the microenvironment can be categorized into two main types: exogenous regulation, where active substances produced by other body tissues and organs are transported to the testis via the endocrine system, indirectly influencing SSC proliferation and differentiation; and endogenous regulation, wherein various factors within the microenvironment directly or indirectly impact SSCs through a network of regulatory mechanisms, affecting their proliferation, differentiation, and metabolism (Yoshida et al., 2007; Ryser et al., 2011).

The vascular system plays a pivotal role in both modes of regulation, as the endocrine factors involved rely on blood circulation. Yoshida et al. (Yoshida et al., 2007) labeled undifferentiated spermatogonial cells in transgenic mice with Ngn3-GFP and Plzf, discovering that undifferentiated spermatogonial cells were predominantly located near the vasculature and mesenchymal stromal cells. In contrast, differentiated spermatogonial cells migrated randomly within the basal layer of the seminiferous epithelium. The proximity of these cells to the testicular mesenchyme suggests that hormones and factors transported via the bloodstream or produced by mesenchymal cells are vital for SSC self-renewal and differentiation. Additionally, these hormones and factors regulate the hypothalamic-pituitary-testicular axis, further influencing SSCs and their microenvironment. Gonadotropin-releasing hormone (GnRH) from hypothalamic neurons stimulates the anterior pituitary to secrete follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which regulate spermatogenesis. LH accelerates testosterone release from interstitial cells, while support cells, expressing both testosterone and FSH receptors, are the primary targets of hormonal signaling, thus mediating spermatogenesis.

Ryser et al. (2011) noted that prepubertal spermatogonia are mostly SSCs with stem cell potential, and prepubertal support cells create a conducive microenvironment for SSC survival while proliferating themselves. Specific genes in prepubertal spermatogonia and support cells, such as Integrin α6 and β1 on spermatogonia and ECM component-producing genes in support cells, facilitate their interaction. It has also been shown (Wright et al., 2011) that a

secreted protein, Upd3, in *Drosophila* activates the JAK/STAT signaling pathway before interacting with the ECM, and that Upd3 interacts with the ECM by binding to the ECM.Upd3, when expressed as a fusion protein (bound to the green fluorescent protein GFP) in *Drosophila in vitro* cell culture, enhances the JAK/STAT activity and positively regulates the binding of ECM to related proteins.

Our studies have shown that GDNF stimulation upregulates genes impacting SSC self-renewal and survival (Oatley et al., 2006; Oatley et al., 2007). After GDNF withdrawal, there is increased activation of STAT3 and Neurog3 genes in spermatogonial cell cultures, with suppression upon re-exposure. Intratesticular transplantation in leucodendron-treated recipient mice revealed an increase in cells capable of regenerating spermatogenesis in cultured populations of undifferentiated spermatogonia after 18 h of GDNF (Oatley et al., 2006). The hypersensitivity of SSCs to GDNF after sustained *in vitro* exposure may enhance colonization efficiency post-transplantation. The increased activation of STAT3 and upregulation of Neurog3 post-GDNF withdrawal correlate with the enhanced colonization efficiency of SSCs.

## Relationship between JAK/STAT pathway and homing migration of spermatogonial stem cells

Homing is an intrinsic characteristic of spermatogonial stem cells (SSCs). During their transition to undifferentiated spermatogonia, gonadotrophs migrate into a stem cell ecotope comprising basement membranes and supporting cells. Transplanted SSCs can navigate into this ecotope and reinitiate spermatogenesis over an extended period (Brinster and Zimmermann, 1994). Research indicates that glial cell line-derived neurotrophic factor (GDNF) plays a role in SSC homing (Dovere et al., 2013; Huleihel et al., 2013; Yang et al., 2013). Prolonged GDNF activity appears to increase the likelihood of SSC colonization, possibly because stress responses enhance SSC homing to accessible ecological niches, thereby influencing colonization efficiency.

Moreover, the CXCL12-CXCR4 signaling pathway is crucial in mouse stem cells for colonization of recipient testes post-transplantation, likely affecting homing and the establishment of stem cell niches. Inhibiting CXCR4 in the adult mouse testis impairs SSC maintenance and leads to germline loss, underscoring CXCL12's role in the mammalian testicular stem cell milieu in regulating SSC homing via CXCR4 signaling (Yang et al., 2013). The CXCL12-CXCR4 axis is known to regulate migration, proliferation, and survival of various cell types (Peled et al., 1999; Arai et al., 2005; Masztalerz et al., 2007; Ni et al., 2016). Inhibition of CXCR4 signaling in primary cultures of undifferentiated spermatogonia leads to detachment from feeder cells, indicative of impaired cell adhesion properties and reflecting SSC homing characteristics post-transplantation (Peled et al., 1999). SSCs lacking CXCR4 expression fail to form donor-derived spermatogonial colonies after transplantation, likely due to reduced migration to the basement membrane, suggesting CXCL12-CXCR4's influence on SSC homing to ecological niches (Masztalerz et al., 2007). Additionally, CXCL12 has been implicated in maintaining hematopoietic stem/progenitor cell quiescence and regulating the JAK/STAT pathway in SSCs (Allard et al., 1995; Arai et al., 2005).

CXCL12 acts as a chemokine for SSCs, with receptor defects reducing colonization efficiency (Tamiya et al., 2011). Both GDNF and CXCL12 promote cobblestone zone formation in SSCs and germline stem cells (GSCs) *in vitro*, as GDNF increases CXCR4 expression (CXCL12's receptor). These factors may act synergistically in SSC homing, with inhibition of these signals decreasing SSC homing efficiency post-transplantation and suggesting their role in vivo SSC homing (Takashima and Shinohara, 2018) (Figure 3).

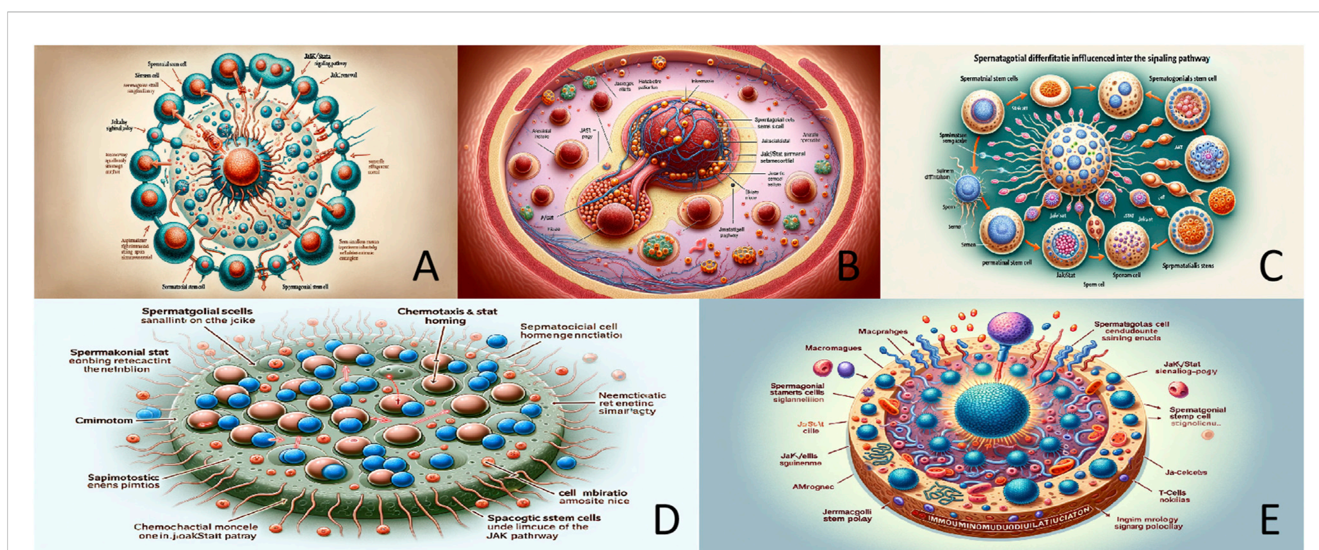


FIGURE 3
Regulation of biological properties of spermatogonial stem cells by JAK/STAT signaling pathway. **(A)** The relationship between the JAK/STAT pathway and spermatogonial stem cell proliferation; **(B)** Relationship between the JAK/STAT signaling pathway and the microenvironment of spermatogonial stem cells; **(C)** Relationship between the JAK/STAT pathway and spermatogonial stem cell tissue differentiation; **(D)** Relationship between JAK/STAT pathway and homing migration of spermatogonial stem cells; **(E)** The JAK/STAT pathway and immune regulation in spermatogonial stem cells.

## Conclusion

This paper synthesizes previous research on the JAK/STAT signaling pathway and spermatogonial stem cells (SSCs), highlighting that these studies predominantly concentrate on the protein expression and genetic material content of JAK/STAT signaling pathway molecules. The role of the JAK/STAT signaling pathway in SSCs and their target cells has been elucidated in two principal areas:

First, the impact of the JAK/STAT pathway in target cells on SSCs, which is instrumental in regulating microenvironmental imbalances and has potential applications in disease treatment and adjuvant therapy. Second, the differentiation, immunomodulation, adaptive changes, and homing of SSCs are all linked to the JAK/STAT signaling pathway:1.Differentiation: Cytokine-activated JAK/STAT signaling contributes to SSC differentiation. 2. Immunomodulation: SSCs foster immune cell proliferation through specific surface antigen expression and cytokine release via the JAK/STAT pathway. 3.Adaptive Changes: SSCs exhibit enhanced survival rates in both *in vivo* and *in vitro* environments when the JAK/STAT pathway is activated. 4.Homing: The JAK/STAT pathway guides SSC migration towards chemokine-releasing cells.

The main stem cell sources for studying the biological properties and JAK/STAT pathway include *Drosophila*, rats, and sheep, with human and large mammalian studies being relatively rare. There is a need to develop a comprehensive theoretical framework for the biological properties and JAK/STAT signaling pathway of SSCs across different species.

SSCs hold considerable promise for clinical applications, particularly in adjuvant therapy and disease treatment. Current tissue engineering research focuses on the differentiation function of SSCs to replace damaged or destroyed cells. However, foundational research on preclinical applications of SSCs is closely linked to the exploration of cellular signaling pathways. This paper contributes to the understanding of SSCs by examining their relationship with the JAK/STAT signaling pathway, offering vital insights into the signaling mechanism and providing theoretical support for further preclinical studies.

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XG: Conceptualization, Writing–original draft, Writing–review and editing. LD: Writing–review and editing. DH: Investigation, Writing–review and editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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