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Review Article

Sperm RNA: a New Class of Fertility Biomarkers for Birds

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ABSTRACT

Leeuwenhoek discovered and named spermatozoa but could not convince the scientific world the potentiality of the sperm. Similarly, transcriptionally quiescent genetic material was marked by a state of tranquil among researchers until the breakthrough of RNA transcript residing inside the sperm. Even though, queries and enigmas highlight throughout sperm research, most reports favor us in thinking about role of these transcripts in fertilization and early embryonic development. Many sperm transcripts have been confirmed as bio–fertility marker in human and other mammalian specie. With this review, we are attempting to summarize spermatozoal work conducted so far in other vertebrate classes and thus representing a potential to serve as bio–fertility marker through such a non–invasive approach in birds.

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INTRODUCTION

Among all the bird species, poultry, the descendent of jungle fowl has the proud history as food animal for serving mankind and also as a model species for research. The modern chicken has evolved through conventional breeding which has dramatically increased the body weight. However, this long term selection for body weight has resulted in decreased fertility rate because of impaired metabolic processes (Hockins et al., 1989). In addition to this, environmental exposures including chemical, thermal and biological agents pose serious threats to reproductive success in both poultry and wild birds.

Male fertility requires the production of an adequate number of normal mature spermatozoa with sufficient motility and the ability to undergo acrosome reaction in order to bind and penetrate the egg membrane for fertilization. Defects in any of these necessary characteristics can lead to male infertility. Therefore, selection of high fertility potential males could be beneficial for poultry breeding and captive breeding programmes of wild birds. Till date only phenotypic traits viz. comb area, testicular weight and semen characteristics are considered for male selection (McGary et al., 2002). Another method of male selection is based on physical and biochemical characterization of semen viz. visualization of sperm motility, semen colour, ejaculate volume, sperm concentration, sperm metabolism and enzymes (Froman et al., 2007). Further, many of these semen tests evaluate a single characteristic of sperm and do not account for the complex process of fertility. The phenotypic traits are not promising and do not guarantee higher male fertility. Hence, unsuitability of above conventional methods for fertility evaluation inspires scientists to explore the reliable sperm based fertility biomarkers.

Recent interest in spermatic RNA has been motivated by the potential which may offer as a diagnostic tool for infertility as evident in human and other mammalian species (Johnson et al., 2011). Transcriptionally silent sperm is thought to comprise genetic material 'DNA' alone and sperm is simply a mediator carries this to the egg. The role of paternal genome in fertilization was not given much importance until the breakthrough of transcript population of about 10–20 fg as mRNA and small non–coding RNA (snc RNA) of varying length in sperm. Different researchers have their own opinion about origin of the traces of these spermatozoal transcripts in a transcriptionally quiescent sperm cell; Kramer and Krawetz (1997) proposed those as remnant untranslated RNAs during spermatogenesis, and are strongly appreciated after observing same spermatozoan transcript in both testes. Uncovering of spermatozoal RNA transcripts and their probable role in poultry and other bird species as already stated among other vertebrates (Goodrich et al., 2007; Gilbert et al., 2007; Lalancette et al., 2008; Yang et al., 2009; Das et al., 2010) may provide important breakthrough in identifying fertility biomarkers. In this review, we discuss the possibilities of using sperm RNAs as fertility/ infertility biomarkers based on the available literature for mammalian and other species because few reports are available on this particular subject on chicken sperm.

SPERM RESEARCH AT A GLANCE

The presence of sperm in semen was first termed as parasitic animal, and thereafter the term spermatozoa were

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coined (Ruestow 1983). Leeuwenhoek then compared sperm as "seed" which implanted in nutrient soil so called "egg". Later his co–discoverer Nicolas Hartsoeker drew the picture of "homunculus" within human sperm (Pinto–correia, 1997). Unfortunately these works couldn't convince science at earlier times and implied these particles as an enormous waste of potential life. However, with subsequent advancement in sperm research the most remarkable achievement in the field of sperm biology happened during the 21st century when functional spermatozoa were raised from bone marrow stem cell (Nayernia, 2006) and the role of sperm RNAs in fertilization and post fertilization was discovered. Before 21st century, it was assumed that sperm facilitate only paternal genome to the egg at the time of fertilization. However, with the advancement in sperm biology research, we now know that sperm not only carry paternal genome but also deliver a variety of RNAs to the egg at the time of fertilization, and those RNAs have specific functions in the fertilization and early embryonic developmental process thereby serving as a diagnostic tool for fertility/ infertility (Johnson et al., 2011).

MORPHOLOGICAL FEATURES AND PACKAGING OF SPERM

Spermiogenesis is a unique process in cytodifferentiation without any further cell division. It is considered as an integral and important part of spermatogenesis in animals. Chicken spermiogenesis constitutes various actions to process round spermatid to an elongated acrosome, stretched mitochondria and slimmer nucleus bearing spermatozoa (Lake, 1956; Nagano, 1962; McIntosh and Porter, 1967; Okamura and Nishiyama, 1976). Where morphology is concerned, chicken sperm are vermiform cells with a maximum width of 0.5–0.7 µm and length of 75–90 µm (Gunawardna and Scott, 1977). They contain a conical acrosome, a slightly bend cylindrical nucleus which extend posteriorly from acrosome to neck region and a helix of 25–30 mitochondria that extends back to annulus, surrounding proximal portion of long flagellum, which account for approximately 84% of the cell's length (Thurston and Hess, 1987).

The maturation from spermatid to spermatozoa delivers not only changes in sperm morphology but also several changes in chromatin structure and function. During chromatin remodeling most somatic histones are replaced by DNA packaging proteins that are specific for germ lines (Caron et al., 2005). Even though the chromatin structure in the sperm nucleus is similar to those somatic cells, organization and packaging is based on looped domains attached at their bases to the nuclear matrix (Ward, 2010). Process of packaging involves various molecular interactions, namely, DNA–DNA, DNA–histone and protein–protein interactions (Roux et al., 2004). A study depicts 85% nucleoprotamines associated with sperm chromatin of mice (Adham et al., 2001), which shows the role of this arginine rich basic protein in packaging. It is thought that sperm packaging is a conserved process among the species mainly because of two reasons: 1) similarity in proteomic sequence of protamine (Nakano et al., 1976, Olivia and Dixon, 1988); and 2) sperm nucleohistones are replaced with nucleoprotamine in many species (Chiva et al., 1987). Fowl protamine is particularly interesting among the vertebrate due to its content of serine residue, possibly which could regulate through reversible post–transcriptional modification such as enzymatic phosphorylation and de–phosphorylation of serine *in vivo* (Louie and Dixon, 1972).

ROLE OF SPERM RNA

First report for the transcript in sperm was an mRNA within the condensed chromatin from the mature sperm nucleus of fern *Scolopendrium* (Rejon et al., 1988). The very next year, Pessot (1989) visualized RNA in mature spermatozoa using the RNase colloidal gold procedure. The first specific RNA to be identified in mature spermatozoa was that of U1 and U2 snRNA (Concha et al., 1993) whereas proto–oncogene *c–myc* was the first mRNA reported (Kumar et al., 1993). Subsequently, human leukocyte antigen (Chiang et al., 1994), β–actin, protamine (Miller et al., 1994) and aromatase (Lambard et al., 2003) mRNAs were observed in spermatozoa.

Presence of specific mRNA zfp59, a zinc finger protein which is usually expressed in post meiotic male germline cells is seen accumulated in the nuclei of rodent spermatozoa, suggest to having a role in DNA organization and condensation during terminal differentiation (Passananti et al., 1995). Adhesive β_1 integrin protein on the surface of ejaculated spermatozoa (Glander and Schaller, 1993) and on spermatogenic cells of human testis (Schaller et al., 1993) may play role in adhesion during fertilization (Rohwedder et al., 1996). Transcript corresponding to round spermatid transcribed human PRM1, PRM2 and TNP2 identified in both epididymal and ejaculate spermatozoa (Wykes et al., 1997) have a role in chromatin condensation. Transcripts of progesterone receptors PR–A and PR–B (Sachdeva et al., 2000), estrogen receptors-α and -β (O'Donnell et al., 2001), leptin receptor at tail region (Jope et al., 2003) and androgen receptor (Solakidi et al., 2005) in ejaculated sperm has been associated with fertility. Presence of testis–specific serine/ threonine kinase 6 (TSSK6) both in the head of elongated spermatid and equatorial segment of the ejaculated spermatozoa of mice (Spirindov et al., 2005; Xu et al., 2007) have the potential to phosphorylate basic protein, thus its role in spermatogenesis and fertility.

Some of spermatozoal transcripts thought to play significant role in fertilization as reported, ERα gene knockout mice are infertile and have poor quality sperm (Eddy et al., 1996). Leptin level in seminal plasma negatively correlated with sperm progressive movement (Abavisani et al., 2011). mRNA of the TSSK6 gene deleted in azoospermia like (DAZL) and Pumilio–2 (PUM2), which are essential for the germ cell development (Fox et al., 2005). Expression of TNP1 and 2 were significantly lower in spermatozoa from asthenozoospermic men compared to normal (Jedrzejczak et al., 2007).

However, most recent researches reported that spermatozoa comprise abundant population of non–coding RNAs (ncRNA) – about 24000 estimated in individual sperm (Krawetz et al., 2011). Sperm–borne micro RNA miR34c (Liu et al., 2012), two siRNA like IGF2 receptor antisense and antisense sequence for *Dickkopf–2* gene (Krawetz, 2005), small RNAs in low copy number from piRNA locus (Kawano et al., 2012) tRNA derived small RNA (Peng et al., 2012) also have specific post fertilization functions. Furthermore, RNA molecules confined in sperm are now considered as key vectors of epigenetic variation

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(Kiani and Rassoulzadegan, 2012). However, very little information is available on this subject on chicken sperm. Hence, transcription profiling of avian sperm may lead to explore their function significance during sperm formation and in early embryonic development. Since, sperm RNAs are the indirect measure of success of spermatogenesis process, they have the potential to serve as an excellent fertility/ infertility biomarkers.

SPERMATOZOAL RNA IN BIRDS

Transcript analysis from avian sperm is challenging as there was no optimized RNA isolation protocol so far. This is because of various reasons; the accessory reproductive organs like lymphatic folds are either in proximity to or are an integral part of the cloaca (Fujihara, 1992). Their secretion along with semen is a primary cause of somatic cell contamination with lymphocytes. Moreover, the reduction in cytoplasmic and nuclear volume to a 3% of initial spermatid cell volume in rooster sperm (Sprando and Russel, 1988) make it difficult in adopting the classical RNA isolation methods (Chomczynski, 1933, Chomczynski and Mackey, 1995).

In chicken, we believe, morphologically normal healthy sperm occupy significantly greater amount of the total RNA than unfit and abnormal counterpart as it is already stated in human (Roudebush et al., 2004). These transcripts may positively correlate to direct fertilization success and early embryonic gene expression and may soon be utilized as marker for male fertility (Johnson et al., 2011). In one of our recent studies, we could amplify protamine transcripts from chicken sperm, which can be used as bio–marker for fertility prediction (Shafeeque et al., 2013).

CONCLUSION

All the transcripts which either represent the remnant of transcript sharing or lack of means of their degradation may have some role in fertilization and early embryonic development in birds. Hopefully a better understanding and characterization of these transcripts by modern technology in avian sperm will provide insight in development of a non–invasive approach to evaluate male fertility. These non–invasive fertility biomarkers will have immense value in captive breeding programs of endangered birds.

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