NEW DEBATE

Cytoplasmic droplets: the good, the bad or just confusing?

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The term cytoplasmic droplets is used to mean two different structures by basic scientists and clinicians. The literature on the presence, position and loss of cytoplasmic droplets and their relationship to infertility in animal species is reviewed. It is proposed that a change in terminology is required: ‘cytoplasmic droplet’ should be used to describe normal droplets associated with functional sperm produced by normal spermatogenesis and ‘excess residual cytoplasm’ is suggested to describe abnormal droplets associated with dysfunctional sperm that are products of faulty spermatogenesis.

Key words: cytoplasmic droplet/excess residual cytoplasm/spermatogenesis

Cytoplasmic droplets: the basic scientist’s view

Despite its discovery by Retzius as early as 1909, the cytoplasmic droplet of spermatozoa remains an enigma. It has been variously known as a kinoplasmic droplet (White et al., 1959; White and Wales, 1961; as it was considered to have something to do with motility: Merton, 1939), a cytoplasmic bead (Hancock, 1956), a protoplasmic droplet (Branton and Salisbury, 1947; Rao and Hart, 1948; Bialy and Smith, 1958; Amann and Almquist, 1962), a cytoplasm droplet (Matousek and Kysilka, 1984) and a plasma droplet (Waberski et al., 1994), but is generally known today as a cytoplasmic droplet. It is a remnant of the germ cell cytoplasm, most of which is phagocytosed by the Sertoli cells, that remains adherent at the neck region of the elongating spermatid when it is shed as a testicular spermatozoon at spermiation during normal spermatogenesis.

In most species studied there is convincing evidence that it migrates along the midpiece from neck to annulus during proximal epididymal transport and remains on the majority of cells in the epididymis (see Cooper and Yeung, 2003). The mechanism of transport has not been elucidated but migration of droplets from the neck of caprine and porcine testicular spermatozoa can be achieved by the application of repeated or sustained centrifugation (Kato et al., 1983, 1984). Peristaltic motions of the epididymal tubule on high concentrations of spermatozoa within the lumen could thus contribute to the migration of the droplet along the tail.

That fewer sperm have droplets in the ejaculates of boars, rams, bulls and goats (Lasley and Bogart, 1944a,b; White et al., 1959; O’Donnell, 1969; Kato et al., 1996) suggests that they are removed around the time of ejaculation. Sperm recovered from the murine uterus after mating also display no cytoplasmic droplets (Merton, 1939). In the bull there are fewer sperm with droplets in the ampulla (Branton and Salisbury, 1947; Rao and Hart, 1948; Matousek and Kysilka, 1980) and seminal vesicle fluid can release droplets from testicular and mature and immature epididymal spermatozoa (Bialy and Smith, 1958; Larson et al., 1980; Matousek and Kysilka, 1980). A haemolytic phospholipid binding protein (PBP) has been identified in the bovine ampulla and seminal vesicles, but not epididymal fluid, which is capable of liberating droplets from mature and immature spermatozoa from several species (Matousek and Kysilka, 1980, 1984). PBP protein appears to be absent from the boar accessory and ampullary glands, which could explain the presence of sperm with droplets in the porcine ejaculate (O’Donnell, 1969). However, the greater retention of droplets on spermatozoa in semen from vesciculectomized boars (Harayama et al., 1996) also argues for a component of seminal vesicle fluid being responsible for shedding the droplet, and fructose has been postulated to be effective in this species (Harayama et al., 1996). However, even in the absence of seminal vesicle secretions, droplets are lost rapidly upon incubation of the sperm-rich fraction of the porcine ejaculate (Kato et al., 1996) that initially contains the same percentage of spermatozoa with droplets as those in the vas deferens (Hancock, 1956).

Retention of ‘true’ cytoplasmic droplets and infertility in domestic species

If the droplet is normally shed then its retention on sperm in the ejaculate may be associated with infertility, and there is considerable evidence that this is the case. Most reports relate to the retention of proximal droplets (at the neck), indicative of a failure of normal epididymal maturation. This occurs in young bulls where success rates of IVF are below normal, despite the selection of spermatozoa, and the situation improves as the male ages (Amann et al., 2000). Further analysis reveals that the reduced cleavage and fertility rates reflect poor passage through hyaluronate swim-up medium and a failure to bind to the zona pellucida (Thundathil...
et al., 2001). Infertility characterized by reduced pregnancy rate and litter size is also associated with retention of the distal droplet (at the annulus) in boars (Waberski et al., 1994), although proximal droplets are also retained in these males. Retained droplets (whether located proximally or distally is not indicated) also hinder binding to the uterine epithelium (Petrunkina et al., 2001). In the mouse, too, infertility in the male is associated with retention of the droplet on sperm in the uterus of females mated to c-ros knockout males (Yeung et al., 2000).

Retention of ‘residual cytoplasm’ on human spermatozoa and infertility

There are many reports indicating that the presence of excess residual cytoplasm on spermatozoa is associated with poor sperm function. For example, the presence of a ‘cytoplasmic droplet’ on human spermatozoa is associated with infertility in men who smoke (Mak et al., 2000) or who suffer varicocele (Zini et al., 2000) and the retention of such a droplet is related to a shorter axoneme (Gergely et al., 1999), poor sperm motility (Zini et al., 1998), abnormal head and midpiece morphology (Huszar and Vigue, 1993; Gomez et al., 1996; Gergely et al., 1999), lower fertilizing capacity (Keating et al., 1997), reduced binding to the zona pellucida (Liu and Baker, 1992; Huszter et al., 1994; Ergur et al., 2002), greater extents of chromatin breaks and DNA damage (Fischer et al., 2003) and increased chromosomal disomies (Kovanci et al., 2001). The mechanisms by which reduced function is exhibited by abnormal sperm retaining excess cytoplasm has been associated with disturbed membrane remodelling (Huszar et al., 1997) and higher extents of lipid peroxidation (Aitken et al., 1994; Huszar and Vigue, 1994; Ollero et al., 2000).

Cytoplasmic droplets: the clinician’s view

The excess residual cytoplasm referred to above as a ‘cytoplasmic droplet’ on human spermatozoa observed in air-dried smears should, according to Menkveld et al. (1990) and WHO (1999) terminology, be termed an ‘abnormal cytoplasmic droplet’ as it occupies >1/2 [to >1/3] of the size of the sperm head in an air-dried, fixed and stained preparation. However, most clinicians regard all observable cytoplasmic droplets as abnormal cytoplasmic droplets. Our recent paper (Cooper et al., 2004) draws attention to the fact that the clinician’s view of cytoplasmic droplets does not accord with that of the basic scientist. First, we demonstrate that the ‘cytoplasmic droplets’ which survive the air-drying technique for human seminal smears are not the ‘cytoplasmic droplets’ discussed above as normal organelles of functional spermatozoa produced by a normal testis from domestic species. Second, we consider them to be ‘excess residual cytoplasm’ that is characteristic of abnormal spermatozoa produced by defective spermatogenesis. Amann et al. (2000) concurs that the cytoplasmic droplets of rams differ from the ‘retained cytoplasm’ of human sperm. Third, ‘true’ cytoplasmic droplets are present on human spermatozoa, particularly motile ones, in wet preparations (see below).

Human cytoplasmic droplets: a revised view

Cooper et al. (2004) identify ‘true’ cytoplasmic droplets on living and well-fixed spermatozoa, 50% of which do not survive the routine air-drying, fixing and staining procedures of routine morphological preparation of semen smears. The remaining droplets could be observed in Papanicolaou-stained smears as small vesicles at the neck of the sperm and were present to a greater extent than what are usually considered ‘cytoplasmic droplets’ by andrology technicians (i.e. abnormal droplets, >1/2 or 1/3 of the sperm head size). Abraham-Peskir et al. (2002) have also identified osmotically-sensitive ‘mid-piece vesicles’ on living human spermatozoa that exceed the percentage of sperm with residual cytoplasm (as identified in smears) in the same sample. We consider these vesicles to be the cytoplasmic droplets we observed in wet and fixed preparations and to be the equivalent of cytoplasmic droplets of domestic species.

In having a cytoplasmic droplet, human spermatozoa are thus similar to other mammalian species, but they differ in its position (at the neck, not annulus) and in its retention in the ejaculate. Although retention of a proximal droplet in the ejaculate of domestic species is related to infertility (see above), this is because it relates to failed maturational translocation along the midpiece. To the extent that cytoplasmic droplets less than 1/2 or 1/3 of the size of the sperm head in air-dried smears are not considered abnormal (WHO, 1999), their presence goes unrecorded and their relationship to fertility or infertility remains unknown. On the other hand, their detection on human spermatozoa recovered from cervical mucus in vitro (Abraham-Peskir et al., 2002) and oviduct in vivo (Mortimer et al., 1982) may indicate that they are normal structural features of functional spermatozoa.

Human cytoplasmic droplets: time for a change in terminology?

We invite comments on the suggestion that a standardized terminology be employed by basic scientists and clinicians alike, so that the well-established term ‘cytoplasmic droplet’ always refers to the normal remnant of cytoplasm on sperm produced by a normal testis (regular in outline but possibly extending the length of the midpiece in wet preparations; small and unstained in stained smears). This necessitates another name for what was generally accepted to be a cytoplasmic droplet of human spermatozoa. We suggest the terminology ‘excess residual cytoplasm’ be used to refer to the large amounts of cytoplasm on sperm produced by imperfect spermatogenesis (irregular in form in both wet and smeared preparations and with stainable content in the latter). This nomenclature associates ‘excess residual cytoplasm’ on ejaculated human spermatozoa with human infertility, as confirmed in many clinical reports, but retains the accepted terminology of cytoplasmic droplet for a normal organelle of a functional spermatozoon. As excess residual cytoplasm-bearing spermatozoa are incapable of undergoing maturation, they should not be termed immature spermatozoa; they are rather immature germ cells that have not undergone normal terminal differentiation.
References


