Review

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Review

The Impact of Testicular Hyperthermia, Its Physiological Relevance and the Ability of Antioxidants to Overcome the Condition

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Abstract: Testicular heat stress is a well described phenomenon that occurs in mammals that possess a scrotum. Different models to induce testicular hyperthermia, such as surgical cryptorchidism, hot water bath, scrotal insulation or increased environmental temperature have all shown that spermatocytes and spermatids are unambiguously affected by high temperature, resulting in poor sperm production weeks later. Furthermore, a body of evidence suggest the involvement of oxidative stress is either a major or contributory pathway, which gives rise to the potential to overcome this condition. Whilst experimental models conclusively show the deleterious effect of testicular heat on sperm quality, the physiological relevance of the work is still debated. Herein we summarise a cohort of studies that report the effect of "season" on sperm quality. The data show season can affect sperm production, motility and morphology depending on where the work was performed. In countries where temperatures drop below zero, there is evidence showing summer conditions tend to improve semen quality. However, in sub-tropical countries, some studies show a decrease in summer, whilst others show no change. Herein we offer a reasonable explanation for this apparent controversy and present a range of antioxidant supplements that may offer some protection against testicular hyperthermia.

Keywords: testicular heat stress; NRF2; heme oxygenase; seasonal variantion; hyperthermia; scrotal heat stress

Different models of testicular hyperthermia but similar outcomes.

The impact of testicular heat-stress has its origins back in 1893, when Joseph Griffiths noticed nobody had tried to "experimentally determine" the observation of John Hunters that testicles fail to reach their full size and produce sperm when retained in the abdomen (cited in 1). To understand the origin of that condition, Griffiths offered two postulates. First, the lack of testis growth was the fault of a "young-testis", being imperfectly formed at the beginning then failing to descend and mature. Second, the main problem is not within testis but rather comes from the environment to which it is exposed. By performing unilateral surgical cryptorchidism in Fox Terriers, Griffiths noted the testis "dwindle to a considerable extent" and were incapable of producing spermatozoa after 6 months[1]. Unbeknownst at that time, this work heralded the beginning of the field of studies in testicular hyperthermia.

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Since then, a diverse range of experimental models have been used, all of which confirm that spermatogenesis is a heat-sensitive process. Such models seek to do one of two things; either prevent the testis from descending to its normal position or overwhelm the ability of the scrotum to regulate testicular temperature. With regards to the latter, strategies include surgical cryptorchid models, mostly performed in laboratory animals (e.g., mice[2, 3], rats[4],[5],[6]rabbits[2],[7] and non-human primates[2,[8]]), or wrapping the scrotum up in woollen cloths or other textile material to hold testis close to the abdomen and prevent their descent or insulation, more commonly used for domestic animals (e.g., rams[9-12] 8,9 bulls[13-21], boars[22], and dogs[23]). In all cases, a decline in sperm concentration, motility and normal morphology was shown. For bulls, one of the most well studied model species, a decline in semen quality begins 5-15 days post-intervention, peaking around 4 weeks later[13-21], suggesting that early precursor cells exposed at the time of heat were sensitive to hyperthermia.

The use of a hot-water bath[24-35] and elevated environmental temperatures[36-42] are examples of models to "overwhelm" the ability of the scrotum to thermoregulate. These models allows the investigation of a range of temperatures and their impact on sperm production. In the case of rabbit[37] and ram[36], increasing the ambient air temperature up to 43.3^{o}C for one hour, showed a significant decline in sperm parameters, particularly motility[37]. When left for longer (32^{o}C for up to one week), a significant decline in all sperm parameters (i.e., motility, morphology and counts) can be seen five weeks later[37]. Notably, semen quality begins to improve eight weeks post intervention, suggesting spermatogonia are not affected by short-term heat duration.

Testicular hyperthermia-induced oxidative stress in mice

To understand how different temperatures affect spermatogenesis, the best available models come from using hot-water baths on anesthetised mice. From this work, it is very clear that the impact on spermatogenesis is directly related to the temperature and time exposure to heat. For example, scrotal heating with water bath at 41°C, 42°C or 43°C for 30 minutes reduces sperm counts by 54%, 76% and 88% respectively[28]. Furthermore, mice that receive higher degrees of testicular hypothermia take longer to recover [28]. Notably, in this same model system, 25 min of testicular exposure to heat in mice lead to increased levels of the lipid peroxidation marker malondialdehyde, suggesting the involvement of oxidative stress[43]. What is intriguing is that whilst in some cases, testicular hypothermia leads to cell death, in other cases, some cells are able to survive and progress through the spermatogenic pathway leading to sperm production (albeit reduced amounts). How this occurs is unknown, but may involve regulation of heme oxygenase 1 (HO-1), which increases within 6 h post heat together with the antioxidant enzymes glutathione S-transferase (GPX; increases 24 h post heat) [43]. Both HO-1 and GPX are known to scavenge free radicals, thereby reducing the level of MDA and by implication, increase cell survivability. Tying into this idea, is the transcription factor, erythroid 2-realted factor 2 (Nrf2). Under normal conditions, newly synthesized Nrf2 associates with Kelch-like ECH-associated protein and is then degraded through the ubiquitin proteosome pathway[44]. However, the presence of excess free radicals, nitric oxide, zinc and alkenals, cysteine residues within Keap1 are modified[44]. The consequence of which leads to translocation of Nrf2 into the nucleus and up-regulation of specific antioxidant genes through binding to the antioxidant response element sequence [44]. Thus, whilst testicular hypothermia leads to aldehyde production, this in turn activates the Nrf2-antioxidant system to help overcome heat-induced oxidative stress. Furthermore, as outline below, addition of zinc (which activates the Nrf2 system) before testicular hyperthermia is a remarkable defence against cell death, implication involvement of this pathway.

Is "heat-stress" physiologically relevant?

The use of testicular-hyperthermia animal models makes it easier to study the impact of heat on sperm quality. However, the question remains as to whether this is a physiologically relevant event, especially in agriculture species and humans? Furthermore, if changes in sperm production are

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'seasonal' this gives rise to the idea that agriculture and potential clinics can use antioxidant therapy to overcome the effect.

The first report of seasonal effects on breeding efficiency in dairy cattle occurred in 1938[45]. Dairy records taken from the University of Nebraska showed more natural "services" were required for conception from May to October (i.e., summer) compared to winter months. This appeared to be consistent throughout the years 1896 to 1934[45], suggesting summer was detrimental to fertility. In 1941, an examination of 11 years of the breeding records from the Louisiana State University showed seasonal differences in the rate of conception[46]. Again, summer months required more services per conception, whilst the best record for rate of conception was during winter[46]. These data pointed to three possible interpretations; either the cow, the bull or both are somehow affected by heat stress. The notion that physiological temperatures could affect bull fertility was then advanced in 1942, when Purdue University reported that, on average, semen volume and motility were lowest in July, August and September (i.e., summer) whilst the average concentration was maximum during April, May and June[47]. Finally, Anderson also showed bull sperm volume and motility declined during summer in Kenya[48] Experimentally, the idea that summer could be causing a drop in semen quality was verified by the purposeful heating of dairy bulls inside chambers at physiologically relevant (32.2°C) temperatures[49]. Such bulls showed clear evidence that a warm environment can lead to a decrease in sperm morphology and motility[49]. In addition, it is clear that reactive oxygen species generation are involved[50].

Despite these early works, the notion that "season" can impact bull fertility does not come without controversy. In deference to the idea that summer is detrimental to semen quality, reports from Cornell University show winter was the poorest season for breeding dairy cattle in Canada and New York State [48, 51], which was later confirmed [52-55]. Furthermore, others have reported season has no-significant effect on bull semen quality [52, 56-58]. To allow for a broader view, we have taken a closer examination of studies that evalulated the effect of season on bull semen parameters (Table 1).

Table 1. An overview of studies that have looked at the effect of "season" on sperm quality in bulls.

# Bulls	Breed	# ejaculates	Motility (%)		Cell counts		Morphology (% defective)		Country	Ave, temp (°C)		Ave Humidity (%)		Ref
			Sum.	Win.	Sum.	Win.	Sum.	Win.		Sum.	Win.	Sum.	Win.	
6	B. Indicus	n/a	55	39	1.2^{9}	1.1^{9}	39	30	Khon kaen, Thailand	36	30	80	63	[54]
51*	Bos Indicus/indicus	ND	NSD**	NSD**	11^{9}	11^{9}	14	10	Sertãozinho, SP, Brazil Uberaba, MG, Brazil	~26	~19	84	51	[56]
13	Bos Taurus	1103	4.2***	3-3.7***	1023/mm3	1015/mm3	14	14	Missouri, USA	24	-3	60	65	[53]
9 bulls (reduced to 2 over time)	Zebu	1049	75	80	600cc3	700cc3	ND	ND	Kenya	27	20	75	60	[59]
137	Bos Taurus	5644	55	56	$13.5\text{-}14^{9}$	$14.5\text{-}15.5^{9}$	ND	ND	Geuth, Canada	26	-3	60	65	[52]

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5	Swamp Buffalo	118	73	75	4.2^{8}	3.6^{8}	10	11	Khon-Khaen, Thailand.	35	33	89	92	[57]
27	16 Bos. Taurus. 11 Bos. Indicus	ND	NSD	NSD	ND	ND	20	12	Dourados, MS, Brazil	29	22	80	75	[60]
10	Bos Taurus	ND	47	55	ND	ND	14	15	Uppsala, Sweden	18	-3	65	94	[61]
5	Bos Taurus	86	40	49	ND	ND	ND	ND	Hafetz-Haim, Israel	31	16	84	46	[62]
52	Bos Taurus	86	ND	ND	ND	ND	12	11	Sweeden	21	17	74	40	[63]
10	Bos Taurus	ND	58	57	1.8^{9}	1.7^{9}	39	27	Zamiba	28	8	45	59	[58]
218	Bos Indicus	ND	68	70	6.6^{8}	5.7^{8}	ND	ND	Brooksville, Florida, USA	32	19	79	73	[55]
11	Bos Taurus	ND	58	51	ND	ND	27	12	Gijon, Span	21	11	75	75	[64]
7	Bos Indicus	142	28%	36.2	4.1^{8}	3.9^{8}	21	30	Nsukka, Nigernia	27	24	83	44	[65]
2	Buffalo bulls	42	ND	ND	ND	ND	27	18	Pantnagar India,	36	22	28	45	[66]
2	Ongole	86	56	55	8.2^{9}	8.5^{9}	ND	ND	Semarang, Indonesia	27	27	81	82	[67]
	Simmental	89	70	70	9^{9}	9.7^{9}	ND	ND						
271	Bos Taurus	ND	ND	ND	ND	ND	27	17	Irene, South Africa	28	20	60	37	[68]
19	Bos Taurus	ND	ND	ND	ND	ND	14	11	Northern USA	~~2~~	~~2~~	21 to 43	5 to -30	[69]
6	5 ejaculates per season	ND	70	70	1.4^{9}	1.6^{9}	9	7	30	21	8	85	84	[70]
21	Bos Taurus	ND	51.5	54.6	ND	ND	14	15	Spain/ Sweden	18	2	78	88	[71]
11	Bos Indicus /Taurus	2558 (1095 *B. indicus*	57	58	1.6^{6}	1.4^{6}	27	16	Araçatuba, SP. Brazil	25	19	83	72	[72]
		1463 *B. taurus*)	51	59	1.2^{6}	1.2^{6}	44	18						
933	Bos Taurus	29170	90	84	ND	ND	3	7	Netherlands	15	10	95	62	[73]

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176	Bos Taurus	8983	82	82	9[9]	~9[9]	ND	ND	Ireland	14	6	83	68	[74]
3	*Bubalus bubalis*	ND	65	64	5.2[9]	3.4[9]	ND	ND	Indonesia	23	23	79	74	[75]
155	*Bos sondaicus*	155	ND	ND	12[6]	14[6]	ND	ND	Townsville, Australia	33	13	76	64	[76]
288 abattoir /21 breeding bulls	Bison	ND	69	44	7.1[8]	5.1[8]	39	43	Alberta, Saskatchewan, Monitoba			10	0	[77]
7	*Bubalus bubalis*	4834	66	68	1.16[9]	1.11[6]	ND	ND	Salon, India	37	25	62	55	[78]
8	Bos. Taurus and crossbred	558	79	81	3.31[9]	10.1[9]	24	5	Nigeria	37	27	87	76	[79]

ND = not determined. NSD = no significant difference, but no values were reported in the origonal pubication. * three different collections stations used. ** reported as no significant difference, but no values given. ***motility scored from 0-5 (with 5 being the highest).

In Table 1, where available, the motility, morphology and cell counts from each study, together with the reported climatic conditions at the time of semen collection are included. If no climatic data were given, this information was then obtained online (weather-and-climate.com) using seasonal averages. Although this table does not list all the works performed in this area, it is very indicative of the results in this field. From this review it is evident there are two variables that need to be considered, both of which appear to explain much of the "contradictory" results around season and semen quality.

Firstly, most of the work was performed at very different locations, with extremely different climatic conditions. For example, in both Kenya and Thailand in which ambient temperature is consistent throughout the year, little variation in semen quality was observed [56, 63]. However, in northern parts of USA [57] and Sweden [80], where winter temperatures drop below zero and summer is quite mild, sperm motility is higher during the latter season.

The most controversial work comes from countries considered sub-tropical, for example Brazil and Australia. Herein, summer temperatures greatly exceed the core body temperature of the bull (~38^{o}C), whilst winter temperatures can drop will below (10-15^{o}C). Based on the experimental work (purposeful heating in a chamber, water bath etc), it would be expected that such temperatures would impact bulls, if season played a role. Indeed, there is clear evidence of semen quality deteriorating in summer in these countries[72]. However, these findings are not consistent, with one major report from Australia, which looked at 11,387 bulls finding no evidence of seasonal variation[81]. In this report, bulls were sourced from all over the country (as opposed to one area). As Australia is so large, the changes in temperature from the north to south are vast, making it difficult to analyse effectively. However, these same authors did note that bulls found in the hottest part of the country (north region) were less likely to pass a semen test. In support of this, we have reported definitive evidence of seasonal variation within bulls taken from central QLD, Australia[82] where temperatures in summer are exceedingly hot.

Emerging evidence shows there are "degrees" of heat-sensitivity within species.

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In a previous study, six bulls were subject to scrotal insulation "by enclosing the scrotum with a sack constructed of insulated material held in place by Velcro fasteners and medical tape" (cited from [83]). The bulls were then subject to the exact same environmental conditions throughout the study. However, after 9-30 days, two animals showed large increases in abnormal spermatozoa (~65 and 69%), three animals had between 47-51% abnormal sperm and one animal had fewer than 24% abnormal forms[14, 83]. As such, there was large differences in bull thermotolerance, suggesting individual bulls can display degrees of thermosensitivity. Supporting this observation, 48 hours scrotal insulation of 4 bulls caused one animal to drop to a staggering 0.5% morphologically normal spermatozoa, whilst bull 2 and 3 dropped to 22% and 29% respectively. However, the 4th bull, despite undergoing the same experimental insult, showed no change in his semen profile, and maintained 82% normal forms for 3 weeks[84]. Finally, in our work, we took 20 bulls and purposefully heated them in a shed. We found large changes in semen quality following intervention. Some bulls dropped to below 50% normal forms, whilst others maintained their baseline 80% average throughout 12 weeks of testing [82]. Thus, it is clear that there is a spectrum of thermo-sensitivity within individual animals.

The idea of thermo-sensitivity is not restricted to bulls, as evidence points to this being a phenomenon in other animal model systems, and we propose all mammals with a scrotum. For example, within boars used for artificial insemination (AI) it has been previously noted that some breeder-lines appeared to have a high "heat-tolerance" [85].

The mechanism of thermo-sensitivity may be due to oxidative stress. Notably, reports show during summer there is an increase in reactive oxygen species within the mitochondria of cells, leading to lipid peroxidation and an imbalance between pro- and anti-oxidants [50, 86]. As such, the level at which each bull expresses their antioxidant capacity may explain why some bulls are more thermotolerant than others. In addition, it would be interesting to integrate the NRf2 pathway to see if differences in the levels of oxidative stress are occurring between bulls to explain this discriminating phenomenon.

Evidence of genetic heritability of "testicular heat-sensitivity"

Although scarce, there is some data suggesting testicular heat-sensitivity could be inherited. As outlined above, within boars used for AI, there is significant variation in sperm production among the genetic lines[85], with some of them showing higher "heat-tolerance" compared to others. For example, while in one lineage high environmental temperatures led to a 5-7% reduction in sperm output, a decrease of 15-20% was observed in two other lineages, despite using the same amount of heat stress[85]. Additionally, in *Drosophila*, the idea of heat-sensitive and heat-tolerant males has also been reported[87]. Heat-sensitive *Drosophila* males, produce no, or a low number of maggots, after a heat event. Remarkably, the "trait" is heritable. Heat-sensitive males produce "heat-sensitive" male offspring[87]. Conversely, heat-tolerant males produce heat-tolerant offspring[87] even when the same female is used. As such, thermo-sensitivity in *Drosophila* is thought to be passed on from sire to son through the Y-chromosome [87].

Testicular heat stress and its relation to male-factor infertility.

Although the impact of testicular hyperthermia is often studied in the context of livestock animals, there is little doubt that it also plays a significant role in humans. Male infertility is a medical condition affecting one in 20 men in the western world[88, 89] and accounts solely, or in a contributory way, for ~50% of couples attending assisted conception facilities/clinics(AC)[90]. Whilst some infertile men have associated conditions, such as (i) varicocele[91-93] (27% of cases) or (ii) cryptorchidism[94-97] (6% of cases), others do not, therefore falling into the category of "idiopathic infertility" – or infertility of unknown aetiology (35% of clinical load). In all cases, these men produce an abnormal semen profile. Their ejaculate contains low sperm counts, low sperm motility, low sperm morphology[98] or (as is often the case) a combination of these phenotypes. In addition, infertile men produce 2-3 times higher levels of DNA damaged spermatozoa compared to their fertile counterparts[99, 100]; a phenomenon that is often associated with oxidative stress. This contributes

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to the problem, as men with high levels of sperm DNA damage (i.e., more than 40% of sperm population) are essentially infertile[99-101].

In clinical trials, many dating back to the 1980s, scrotal cooling has been shown to improve semen quality and natural pregnancy rates. For example, trials performed in 25 infertile men showed that in 18 (70%), semen parameters improved with scrotal cooling[102]. Furthermore, six (24%) went on to naturally conceive during the 14-week scrotal cooling regime, despite the fact they had been "trying" to conceive for 3-8 years previously[103-105]. To put this into perspective, if a couple has not conceived within 2 years of trying, their chance of conception thereafter is less than 2%[106]. A second trial involving 64 men, showed improvements in semen parameters in 66% of cases and a natural pregnancy rate of 27%[107] within 16 weeks of scrotal cooling. This was statistically significant as the background pregnancy rate (in this case, men with poor semen quality that pulled out of the trial after two weeks) was 5%[107]. However, if men diagnosed with azoospermia (produce no sperm in their ejaculate) or severe oligozoospermia (<1 million sperm/mL in ejaculate) are removed from the analysis, this increases the pregnancy success rate to 50% [107] against a background rate of 10%. Three further studies have looked at pregnancy rates following scrotal cooling with remarkable outcomes (24%[107], 27%[108] and 14% on a background rate of less than 5% in men with history of reduced fertility of at least 3 years). Moreover, these studies were done over 14, 16 and 8 weeks, respectively. Considering sperm parameters take 4-8 weeks to improve with scrotal cooling, this leaves only an one- to two-month window to achieve a pregnancy, which is a remarkable success rate. As such, it is clear that in around 40-80% of infertile/subfertile men, scrotal heat stress is a physiological phenomenon that can be overcome.

Overcoming the impact of testicular hyperthermia.

Dietary supplementation to overcome heat-stress has typically used an antioxidant approach as a major course of amelioration. However, the contribution of oxidative stress to male fertility has recently come under heavy scrutiny mainly due to the limitations and drawbacks of various "probes" used to measure ROS. For example, many reports have used tetrazolium salts (often used to report ROS in sperm) or lucigenin to suggested sperm produce copious amounts of free radicals. However, these assays are actually measuring cytochrome-based enzymes including cytochrome P450-reductase [101] and cytochrome b5-reductase (Cb5R) [102]. Thus, it is likely that such reports simply measure sperm with excess cytoplasm or increased poor morphology. Along similar lines, the use of Luminol-HRP should be avoided, as the assay has indiscriminate recognition of reduced compounds (for example, NADH) and several free radicals making it impossible to determine what is being measured [118]. In contrast, when better discriminatory problems such as 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo [1,2-a] pyrazine-3-one (MCLA) [109] and the electron spin method [100] are used, there is again no evidence that sperm generate large amounts of free radicals.

A better approach to demonstrate whether oxidative stress is a player in testicular hyperthermia is to investigate if supplementation can limit the effect of heat stress. We focused on studies that reported germ cell parameters, sperm quality and pregnancy rates, following heat stress with and without supplementation, rather than those who attempt to biochemically measure ROS through many of the assays aforementioned which are clearly obfuscated in terms of their interpretation. Although many compounds show a significant improvement in sperm counts, the biological relevance of these observations needs to be considered. In most cases, the average decrease following treatment was modest (<10%) [110, 111] [112], [113], [114], [115] [116] despite being statistically significant. However, there have been some exceptional outcomes.

Mice given phytochemicals showed a significantly lower amount of abnormal seminiferous tubules 48 hours after a 15-min exposure to testicular hyperthermia at 41°C. [117] Furthermore, sperm density and motility of treated mice were not significantly different from control mice 7 days later. Phytochemicals appears to work by increasing the expression of Heat shock proteins (HSP) HSPa1a, Hspa1l and Hspa2, together with the transcription factor Heat-shock factor 1. HSP have shown to play an anti-apoptotic role, which may explain phytochemically treated mice able to withstand hyperthermic shock [117]. A second model involved the use of rats, in which males surgically made

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unilateral cryptorchid received xanthine-oxidase inhibitors. After 7 days, the treated group showed significantly higher testicular weights and decrease in apoptotic cells. Furthermore, treated testis showed presence of near-normal testis histology, whereas cryptorchid had lost the majority of round and spermatocytes [118]. These data suggested that a major mechanism by which high temperature leads to apoptosis or poor sperm formation may be through Xanthine-Xanthine oxidase and, in particular, superoxide anion production. Finally, one of the most remarkable protective effects against heat-shock has been seen through administration of zinc. Firstly, mice were given zinc supplements for one month prior to heat stress, which significantly increased the level of testicular zinc. Then, following heat stress (40^{o}C, 2 h daily) for eight days, it was apparent the preservation of most of the germ cells present in the tubules.[119] In proof of concept, zinc supplementation in a similar model also led to an improvement in the number of foetuses born[120]. However, one of the main concerns with this study, is control mice given zinc (without heat stress) showed a reduction in the number of foetuses compared to non-supplemented control mice (40 pups vs 120 pups)[120]. As such, zinc supplementation whilst protects against heat stress must be used with caution as it can decrease the overall birth rates.

Concluding remarks.

Several sources of environmental stress have been suggested to affect semen quality, yet none of them hold up the rigour of scrotal hyperthermia. Although we still do not understand the biochemical mechanisms nor the reason why male-precursor germ cells need to remain at or below 34°C, it is clear that this must be the case. Once temperatures exceed 34^{o}C, spermatocytes tend to undergo apoptosis, whilst spermatids continue to develop into misshapen spermatozoa. Yet the environment may not be the end of the story. The emerging role of genetics, oxidative stress and expression levels of different proteins/enzyme will likely explain the concept of "thermo-sensitivity". The biggest "hint" in terms of causation, is the case of *Drosophila*, where heat-sensitive males produce other heat-sensitive males. Further work to understand the mechanism of testicular heat stress, and answer the age-old question of why the scrotum exists? will certainly be useful not just from a medical point of view, but also in the agriculture industry to identify males that are heat-resistant.

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References

1. J. Griffiths, The structural changes in the testicle of the dog when it is replaced within the abdominal cavity, *Journal of anatomy and physiology*, 27 **1893** 482.481.
2. Z.H. Zheng, Q. Tian, J.P. He, J.L. Yuan, S.H. Yang, J.L. Liu, Comparative transcriptome analysis of experimental cryptorchidism: of mice and cynomolgus monkeys, *Physiol Genomics*, 54 **2022** 187-195.
3. A. Wanta, K. Noguchi, T. Sugawara, K. Sonoda, S. Duangchit, T. Wakayama, Expression of Protein Markers in Spermatogenic and Supporting Sertoli Cells Affected by High Abdominal Temperature in Cryptorchidism Model Mice, *J Histochem Cytochem*, 71 **2023** 387-408.
4. J.L. Yuan, Y.T. Zhang, Y. Wang, Increased apoptosis of spermatogenic cells in cryptorchidism rat model and its correlation with transforming growth factor beta type II receptor, *Urology*, 75 **2010** 992-998.
5. R.A. Aldahhan, P.G. Stanton, H. Ludlow, D.M. de Kretser, M.P. Hedger, Experimental Cryptorchidism Causes Chronic Inflammation and a Progressive Decline in Sertoli Cell and Leydig Cell Function in the Adult Rat Testis, *Reprod Sci*, 28 **2021** 2916-2928.
6. G.D. Sagaradze, N.A. Basalova, V.I. Kirpatovsky, D.A. Ohobotov, O.A. Grigorieva, V.Y. Balabanyan, A.A. Kamalov, A.Y. Efimenko, Application of rat cryptorchidism model for the evaluation of mesenchymal stromal cell secretome regenerative potential, *Biomed Pharmacother*, 109 **2019** 1428-1436.
7. R.M. Vigueras-Villasenor, T. Jimenez Cabrera, M. Chavez Saldana, F. Jimenez Trejo, O. Cuevas Alpuche, J.C. Rojas-Castaneda, Epigallocatechin-3-gallate protects the testis from damage generated by experimental cryptorchidism in rabbits, *Histol Histopathol*, 34 **2019** 931-942.

8. X.C. Zhou, X.B. Han, Z.Y. Hu, R.J. Zhou, Y.X. Liu, Expression of Hsp70-2 in unilateral cryptorchid testis of rhesus monkey during germ cell apoptosis, *Endocrine*, 16 **2001** 89-95.
9. R.W. Phillips, Observations on the Spermatozoa of the Ram and Their Application to the Determination of Fertility, University of Missouri--Columbia, 1931.
10. R.W. Phillips, F.F. McKenzie, The thermo-regulatory function and mechanism of the scrotum, **1934**.
11. C.C. Júnior, C. Lucci, V. Peripolli, A. Silva, A. Menezes, S. Morais, M. Araújo, L. Ribeiro, R. Mattos, C. McManus, Effects of testicle insulation on seminal traits in rams: preliminary study, *Small Ruminant Research*, 130 **2015** 157-165.
12. T. Hamilton, G.E. Duarte, J.A. Visintin, M. Assumpcao, Immunolocalization of antioxidant enzymes in testis of rams submitted to long-term heat stress, *Zygote*, 27 **2019** 432-435.
13. A. Ross, K. Entwistle, The effect of scrotal insulation on spermatozoal morphology and the rates of spermatogenesis and epididymal passage of spermatozoa in the bull, *Theriogenology*, 11 **1979** 111-129.
14. C. Vogler, R. Saacke, J. Bame, J. Dejarnette, M. McGilliard, Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen, *Journal of Dairy Science*, 74 **1991** 3827-3835.
15. A.D. Barth, P.A. Bowman, The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls, *The Canadian Veterinary Journal*, 35 **1994** 93.
16. J. Kastelic, R. Cook, G. Coulter, R. Saacke, Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull, *Theriogenology*, 45 **1996** 935-942.
17. L.F. Brito, A.E. Silva, R.T. Barbosa, M.M. Unanian, J.P. Kastelic, Effects of scrotal insulation on sperm production, semen quality, and testicular echotexture in Bos indicus and Bos indicus× Bos taurus bulls, *Animal reproduction science*, 79 **2003** 1-15.
18. L.D. Newton, J.P. Kastelic, B. Wong, F. Van der Hoorn, J. Thundathil, Elevated testicular temperature modulates expression patterns of sperm proteins in Holstein bulls, *Molecular Reproduction and Development: Incorporating Gamete Research*, 76 **2009** 109-118.
19. M.B. Rahman, L. Vandaele, T. Rijsselaere, D. Maes, M. Hoogewijs, A. Frijters, J. Noordman, A. Granados, E. Dernelle, M. Shamsuddin, Scrotal insulation and its relationship to abnormal morphology, chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls, *Theriogenology*, 76 **2011** 1246-1257.
20. S. Menegassi, G. Pereira, E. Dias, M. Rocha, H. Carvalho, C. Koetz Jr, E. Oberst, J. Barcellos, Infrared thermography as a noninvasive method to assess scrotal insulation on sperm production in beef bulls, *Andrologia*, 50 **2018** e12904.
21. G.R. Pereira, F.L. de Lazari, P.F. Dalberto, C.V. Bizarro, E.R. Sontag, C.K. Junior, S.R.O. Menegassi, J.O.J. Barcellos, I.C. Bustamante-Filho, Effect of scrotal insulation on sperm quality and seminal plasma proteome of Brangus bulls, *Theriogenology*, 144 **2020** 194-203.
22. J.J. Parrish, K.L. Willenburg, K.M. Gibbs, K.B. Yagoda, M.M. Krautkramer, T.M. Loether, F. Melo, Scrotal insulation and sperm production in the boar, *Mol Reprod Dev*, 84 **2017** 969-978.
23. H. Henning, C. Masal, A. Herr, K. Wolf, C. Urhausen, A. Beineke, M. Beyerbach, S. Kramer, A.R. Gunzel-Apel, Effect of short-term scrotal hyperthermia on spermatological parameters, testicular blood flow and gonadal tissue in dogs, *Reprod Domest Anim*, 49 **2014** 145-157.
24. G. Waites, B. Setchell, Effect of local heating on blood flow and metabolism in the testis of the conscious ram, *Reproduction*, 8 **1964** 339-349.
25. B. Setchell, J. Voglmayr, N. Hinks, The effect of local heating on the flow and composition of rete testis fluid in the conscious ram, *Reproduction*, 24 **1971** 81-89.
26. Y. Lue, A.P. Sinha Hikim, C. Wang, M. Im, A. Leung, R.S. Swerdloff, Testicular heat exposure enhances the suppression of spermatogenesis by testosterone in rats: the "two-hit" approach to male contraceptive development, *Endocrinology*, 141 **2000** 1414-1424.
27. B. Setchell, L. Ploen, E. Ritzen, Reduction of long-term effects of local heating of the testis by treatment of rats with a GnRH agonist and an anti-androgen, *REPRODUCTION-CAMBRIDGE-*, 122 **2001** 255-263.
28. B.O. Reid, K.A. Mason, H.R. Withers, J. West, Effects of hyperthermia and radiation on mouse testis stem cells, AACR, 1981.
29. B.J. Sailer, Sarkar, Linda J, Bjordahl, Janet A, Jost, Lorna K, Evenston, Donald P, Effects of heat stress on mouse testicular cells and sperm chromatin structure, *Journal of Andrology*, 18 **1997** 294-301.

30. M. Pérez-Crespo, B. Pintado, A. Gutiérrez-Adán, Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice, *Molecular Reproduction and Development: Incorporating Gamete Research*, 75 **2008** 40-47.
31. A. Hasani, A. Khosravi, K. Rahimi, A. Afshar, F. Fadaei-Fathabadi, A. Raoofi, P. Raee, F. Aghajanpour, A. Aliaghaei, S. Abdi, Photobiomodulation restores spermatogenesis in the transient scrotal hyperthermia-induced mice, *Life Sciences*, 254 **2020** 117767.
32. S. Ilkhani, A. Moradi, A. Aliaghaei, M. Norouzian, S. Abdi, E. Rojhani, A. Ebadinejad, E. Sajadi, M.A. Abdollahifar, Spatial arrangement of testicular cells disrupted by transient scrotal hyperthermia and subsequent impairment of spermatogenesis, *Andrologia*, 52 **2020** e13664.
33. A. Khosravi, A. Hasani, P. Behnam, A. Piryaei, M. Pirani, A. Aliaghaei, P. Raee, S. Abdi, F.F. Fathabadi, M.A. Abdollahifar, An effective method for establishing animal models of azoospermia and oligospermia, *Andrologia*, 53 **2021** e14095.
34. S. Ziaeipour, A. Piryaei, A. Aliaghaei, H. Nazarian, P. Naserzadeh, V. Ebrahimi, S. Abdi, F. Shahi, H. Ahmadi, F.F. Fathabadi, Chronic scrotal hyperthermia induces azoospermia and severe damage to testicular tissue in mice, *Acta Histochemica*, 123 **2021** 151712.
35. M. Rao, X.L. Zhao, J. Yang, S.F. Hu, H. Lei, W. Xia, C.H. Zhu, Effect of transient scrotal hyperthermia on sperm parameters, seminal plasma biochemical markers, and oxidative stress in men, *Asian J Androl*, 17 **2015** 668-675.
36. R. Dutt, P.T. Hamm, Effect of exposure to high environmental temperature and shearing on semen production of rams in winter, *Journal of Animal Science*, 16 **1957** 328-334.
37. A.S. El-Sheikh, L. Casida, Motility and fertility of spermatozoa as affected by increased ambient temperature, *Journal of Animal Science*, 14 **1955** 1146-1150.
38. D.C. Meyerhoeffer, R.P. Wettemann, S.W. Coleman, M.E. Wells, Reproductive criteria of beef bulls during and after exposure to increased ambient temperature, *J Anim Sci*, 60 **1985** 352-357.
39. X. Fan, H. Xi, Z. Zhang, Y. Liang, Q. Li, J. He, Germ cell apoptosis and expression of Bcl-2 and Bax in porcine testis under normal and heat stress conditions, *Acta Histochem*, 119 **2017** 198-204.
40. G.M.J. Costa, S. Lacerda, A.F.A. Figueiredo, M.C. Leal, J.V. Rezende-Neto, L.R. Franca, Higher environmental temperatures promote acceleration of spermatogenesis in vivo in mice (Mus musculus), *J Therm Biol*, 77 **2018** 14-23.
41. J.P. Kastelic, R.E. Wilde, A. Bielli, P. Genovese, G. Rizzoto, J. Thundathil, Hyperthermia is more important than hypoxia as a cause of disrupted spermatogenesis and abnormal sperm, *Theriogenology*, 131 **2019** 177-181.
42. Y.M. El-Gindy, S.M. Zahran, M.H. Ahmed, A.M. Ali, A.Z. Mohamed, S.A. Morshedy, Counteract severe heat stress by including different forms of zinc in the rabbit bucks' diet, *Sci Rep*, 13 **2023** 12987.
43. C. Paul, A.A. Murray, N. Spears, P.T. Saunders, A single, mild, transient scrotal heat stress causes DNA damage, subfertility and impairs formation of blastocysts in mice, *Reproduction*, 136 **2008** 73.
44. A. Kobayashi, M.-I. Kang, H. Okawa, M. Ohtsuji, Y. Zenke, T. Chiba, K. Igarashi, M. Yamamoto, Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2, *Molecular and cellular biology*, 24 **2004** 7130-7139.
45. R.F. Morgan, H. Davis, Influence of age of dairy cattle and season of the year on the sex ratio of calves and services required for conception, **1938**.
46. D. Seath, C.H. Staples, Some factors influencing the reproductive efficiency of Louisiana herds, *J. Dairy Sci*, 24 **1941** 510.
47. R. Erb, F. Andrews, J. Hilton, Seasonal variation in semen quality of the dairy bull, *Journal of dairy science*, 25 **1942** 815-826.
48. E. Mercier, G. Salisbury, Fertility level in artificial breeding associated with season, hours of daylight, and the age of cattle, *Journal of Dairy Science*, 30 **1947** 817-826.
49. R. Casady, R. Myers, J. Legates, The effect of exposure to high ambient temperature on spermatogenesis in the dairy bull, *journal of Dairy Science*, 36 **1953** 14-23.
50. L. Capela, I. Leites, R. Romão, L. Lopes-da-Costa, R.M.L.N. Pereira, Impact of Heat Stress on Bovine Sperm Quality and Competence, *Animals*, 12 **2022** 975.
51. E. Mercier, G. Salisbury, Seasonal variations in hours of daylight associated with fertility level of cattle under natural breeding conditions, *Journal of Dairy Science*, 30 **1947** 747-756.

52. M. Mathevon, M. Buhr, J. Dekkers, Environmental, management, and genetic factors affecting semen production in Holstein bulls, *Journal of dairy science*, 81 **1998** 3321-3330.
53. E.W. Swanson, H. Herman, Seasonal variation in semen quality of some Missouri dairy bulls, *Journal of Dairy Science*, 27 **1944** 303-310.
54. T. Nongbua, A. Utta, N. Am-In, J. Suwimonteerabutr, A. Johannisson, J.M. Morrell, Effects of season and single layer centrifugation on bull sperm quality in Thailand, *Asian-Australasian journal of animal sciences*, 33 **2020** 1411.
55. M. Fields, W. Burns, A. Warnick, Age, season and breed effects on testicular volume and semen traits in young beef bulls, *Journal of Animal Science*, 48 **1979** 1299-1304.
56. L. Brito, A. Silva, L. Rodrigues, F. Vieira, L. Deragon, J. Kastelic, Effects of environmental factors, age and genotype on sperm production and semen quality in Bos indicus and Bos taurus AI bulls in Brazil, *Animal reproduction science*, 70 **2002** 181-190.
57. S. Koonjaenak, V. Chanatinart, S. Aiumlamai, T. Pinyopumimintr, H. Rodriguez-Martinez, Seasonal variation in semen quality of swamp buffalo bulls (Bubalus bubalis) in Thailand, *Asian journal of andrology*, 9 **2007** 92-101.
58. G. Igboeli, A. Rakha, Seasonal changes in the ejaculate characteristics of Angoni (short horn zebu) bulls, *Journal of animal science*, 33 **1971** 651-654.
59. J. Anderson, The periodicity and duration of oestrus in Zebu and grade cattle, *The Journal of Agricultural Science*, 34 **1944** 57-68.
60. M. Nichi, P. Bols, R.M. Züge, V.H. Barnabe, I. Goovaerts, R.C. Barnabe, C.N.M. Cortada, Seasonal variation in semen quality in Bos indicus and Bos taurus bulls raised under tropical conditions, *Theriogenology*, 66 **2006** 822-828.
61. S. Valeanu, A. Johannisson, N. Lundeheim, J. Morrell, Seasonal variation in sperm quality parameters in Swedish red dairy bulls used for artificial insemination, *Livestock Science*, 173 **2015** 111-118.
62. E. Malama, Y. Zeron, F. Janett, M. Siuda, Z. Roth, H. Bollwein, Use of computer-assisted sperm analysis and flow cytometry to detect seasonal variations of bovine semen quality, *Theriogenology*, 87 **2017** 79-90.
63. L. Söderquist, L. Janson, M. Håård, S. Einarsson, Influence of season, age, breed and some other factors on the variation in sperm morphological abnormalities in Swedish dairy AI bulls, *Animal Reproduction Science*, 44 **1996** 91-98.
64. M. Sabés-Alsina, A. Johannisson, N. Lundeheim, M. Lopez-Bejar, J. Morrell, Effects of season on bull sperm quality in thawed samples in northern Spain, *Veterinary Record*, 180 **2017** 251-251.
65. G. Igboeli, L. Nwakalor, B. Orji, G. Onuora, Seasonal variation in the semen characteristics of Muturu (Bos brachyceros) bulls, *Animal Reproduction Science*, 14 **1987** 31-38.
66. M. Sharma, Y. Bhat, N. Sharma, A. Singh, Comparative study of seasonal variation in semen characteristics of buffalo bull, *J. Entomol. Zool. Stud*, 6 **2018** 52-109.
67. N. Isnaini, S. Wahjuningsih, E. Adhitama, Seasonal effects on semen quality of Ongole crossbred and Simmental bulls used for artificial insemination, *Livest. Res. Rural Dev*, 31 **2019** 16.
68. D. Vilakazi, E. Webb, Effect of age and season on sperm morphology of Friesland bulls at an artificial insemination centre in South Africa, *South African Journal of Animal Science*, 34 **2004** 62-69.
69. V. Sekoni, B. Gustafsson, Seasonal variations in the incidence of sperm morphological abnormalities in dairy bulls regularly used for artificial insemination, *British Veterinary Journal*, 143 **1987** 312-317.
70. A. Seifi-Jamadi, M. Zhandi, H. Kohram, N.L. Luceño, B. Leemans, E. Henrotte, C. Latour, K. Demeyere, E. Meyer, A. Van Soom, Influence of seasonal differences on semen quality and subsequent embryo development of Belgian Blue bulls, *Theriogenology*, 158 **2020** 8-17.
71. M. Sabés-Alsina, N. Lundeheim, A. Johannisson, M. López-Béjar, J. Morrell, Relationships between climate and sperm quality in dairy bull semen: A retrospective analysis, *Journal of Dairy Science*, 102 **2019** 5623-5633.
72. M. Koivisto, M. Costa, S.H.V. Perri, W. Vicente, The effect of season on semen characteristics and freezability in Bos indicus and Bos taurus bulls in the southeastern region of Brazil, *Reproduction in Domestic Animals*, 44 **2009** 587-592.
73. N. Llamas-Luceño, M. Hostens, E. Mullaart, M. Broekhuijse, P. Lonergan, A. Van Soom, High temperature-humidity index compromises sperm quality and fertility of Holstein bulls in temperate climates, *Journal of dairy science*, 103 **2020** 9502-9514.

74. E.M. Murphy, A.K. Kelly, C. O'Meara, B. Eivers, P. Lonergan, S. Fair, Influence of bull age, ejaculate number, and season of collection on semen production and sperm motility parameters in Holstein Friesian bulls in a commercial artificial insemination centre, *Journal of animal science*, 96 **2018** 2408-2418.
75. N. Isnaini, T. Harsi, D. Maharani, Seasonal effect on semen characteristics of Murrah buffalo bulls raised under tropical climate, *Jurnal Kedokteran Hewan September*, 13 **2019** 73-75.
76. C. McCool, K. Entwistle, Reproductive function in the Australian Swamp buffalo bull: Age effects and seasonal effects, *Theriogenology*, 31 **1989** 583-594.
77. L. Helbig, M. Woodbury, J. Haigh, J. Collins, A. Barth, The seasonal fertility of North American bison (Bison bison) bulls, *Animal reproduction science*, 97 **2007** 265-277.
78. R. Tiwari, G. Mishra, M. Shukla, R. Singh, S. Saxena, M. Siddiqui, Seasonal variations in semen production of Murrah buffalo bulls, *Indian J. Anim. Reprod*, 32 **2011** 52-54.
79. P. Rekwot, A. Voh Jr, E. Oyedipe, G. Opaluwa, V. Sekoni, P. Dawuda, Influence of season on characteristics of the ejaculate from bulls in an artificial insemination centre in Nigeria, *Animal Reproduction Science*, 14 **1987** 187-194.
80. J. Anderson, Seasonal variation in the reproductive capacity of the bull, *The Journal of Agricultural Science*, 35 **1945** 184-196.
81. A. Barth, The use of bull breeding soundness evaluation to identify subfertile and infertile bulls, *Animal*, 12 **2018** s158-s164.
82. J.K. Netherton, B.R. Robinson, R.A. Ogle, A. Gunn, A.I.S.B. Villaverde, K. Colyvas, C. Wise, T. Russo, A. Dowdell, M.A. Baker, Seasonal variation in bull semen quality demonstrates there are heat-sensitive and heat-tolerant bulls, *Scientific Reports*, 12 **2022** 1-11.
83. C. Vogler, J. Bame, J. DeJarnette, M. McGilliard, R. Saacke, Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine, *Theriogenology*, 40 **1993** 1207-1219.
84. A. Walters, R. Saacke, R. Pearson, F. Gwazdauskas, The incidence of apoptosis after IVF with morphologically abnormal bovine spermatozoa, *Theriogenology*, 64 **2005** 1404-1421.
85. W.L. Flowers, Genetic and phenotypic variation in reproductive traits of AI boars, *Theriogenology*, 70 **2008** 1297-1303.
86. I. Belhadj Slimen, T. Najar, A. Ghram, M. Abdrrabba, Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review, *Journal of animal physiology and animal nutrition*, 100 **2016** 401-412.
87. C. Rohmer, J.R. David, B. Moreteau, D. Joly, Heat induced male sterility in Drosophila melanogaster: adaptive genetic variations among geographic populations and role of the Y chromosome, *Journal of Experimental Biology*, 207 **2004** 2735-2743.
88. M.M. Matzuk, D.J. Lamb, The biology of infertility: research advances and clinical challenges, *Nat Med*, 14 **2008** 1197-1213.
89. R.I. McLachlan, D.M. de Kretser, Male infertility: the case for continued research, *Med J Aust*, 174 **2001** 116-117.
90. A. Agarwal, A. Mulgund, A. Hamada, M.R. Chyatte, A unique view on male infertility around the globe, *Reproductive biology and endocrinology*, 13 **2015** 1-9.
91. H. Hosseinifar, M. Sabbaghian, D. Nasrabadi, T. Modarresi, A.V.T. Dizaj, H. Gourabi, M.A.S. Gilani, Study of the effect of varicocelectomy on sperm proteins expression in patients with varicocele and poor sperm quality by using two-dimensional gel electrophoresis, *Journal of assisted reproduction and genetics*, 31 **2014** 725-729.
92. A. Zini, A. Blumenfeld, J. Libman, J. Willis, Beneficial effect of microsurgical varicocelectomy on human sperm DNA integrity, *Human Reproduction*, 20 **2005** 1018-1021.
93. L. Lund, K. Nielsen, Varicocele testis and testicular temperature, *British journal of urology*, 78 **1996** 113-115.
94. H. Yavetz, B. Harash, G. Paz, L. Yogev, A. Jaffa, J. Lessing, Z. Homonnai, Cryptorchidism: incidence and sperm quality in infertile men, *Andrologia*, 24 **1992** 293-297.
95. S. Taskinen, O. Hovatta, S. Wikstrom, Early treatment of cryptorchidism, semen quality and testicular endocrinology, *The Journal of urology*, 156 **1996** 82-84.
96. E. Pinart, S. Sancho, M. Briz, S. Bonet, N. García, Characterization of the semen quality of postpuberal boars with spontaneous unilateral abdominal cryptorchidism on the right side, *Animal reproduction science*, 55 **1999** 269-278.
97. P.A. Lee, M.T. Coughlin, Fertility after bilateral cryptorchidism, *Hormone Research in Paediatrics*, 55 **2001** 28-32.

98. F. Tüttelmann, C. Ruckert, A. Röpke, Disorders of spermatogenesis, *medizinische genetik*, 30 **2018** 12-20.
99. J.K. Voglmayr, B.P. Setchell, I.G. White, The effects of heat on the metabolism and ultrastructure of ram testicular spermatozoa, *J Reprod Fertil*, 24 **1971** 71-80.
100. B.P. Setchell, The parkes lecture heat and the testis, *Reproduction*, 114 **1998** 179-194.
101. M. Spanò, J.P. Bonde, H.I. Hjøllund, H.A. Kolstad, E. Cordelli, G. Leter, D.F.P.P.S. Team, Sperm chromatin damage impairs human fertility, *Fertility and sterility*, 73 **2000** 43-50.
102. A. Zorgniotti, A. Sealfon, A. Toth, Chronic scrotal hypothermia as a treatment for poor semen quality, *The Lancet*, 315 **1980** 904-906.
103. B. Baccetti, E. Strehler, S. Capitani, G. Collodel, M. De Santo, E. Moretti, P. Piomboni, R. Wiedeman, K. Sterzik, The effect of follicle stimulating hormone therapy on human sperm structure (Notulae seminologicae 11), *Human reproduction (Oxford, England)*, 12 **1997** 1955-1968.
104. Z. Ben-Rafael, J. Farhi, D. Feldberg, B. Bartoov, M. Kovo, F. Eltes, J. Ashkenazi, Follicle-stimulating hormone treatment for men with idiopathic oligoteratoasthenozoospermia before in vitro fertilization: the impact on sperm microstructure and fertilization potential, *Fertility and sterility*, 73 **2000** 24-30.
105. B. Baccetti, P. Piomboni, E. Bruni, S. Capitani, L. Gambera, E. Moretti, K. Sterzik, E. Strehler, Effect of follicle-stimulating hormone on sperm quality and pregnancy rate, *Progressive*, 8 **2004** 12.14-12.11.
106. C. Gnoth, D. Godehardt, E. Godehardt, P. Frank-Herrmann, G. Freundl, Time to pregnancy: results of the German prospective study and impact on the management of infertility, *Human reproduction*, 18 **2003** 1959-1966.
107. A.W. Zorgniotti, M.S. Cohen, A.I. Sealfon, Chronic scrotal hypothermia: results in 90 infertile couples, *The Journal of urology*, 135 **1986** 944-947.
108. A.W. Zorgniotti, A.I. Sealfon, Scrotal Hypothermia: New theraphy for poor semen, *Urology*, 23 **1984** 439-441.
109. E. de Lamirande, A. Harakat, C. Gagnon, Human sperm capacitation induced by biological fluids and progesterone, but not by NADH or NADPH, is associated with the production of superoxide anion, *J Androl*, 19 **1998** 215-225.
110. A.M. Shahat, J.C. Thundathil, J.P. Kastelic, Melatonin improves testicular hemodynamics and sperm quality in rams subjected to mild testicular heat stress, *Theriogenology*, 188 **2022** 163-169.
111. P. Zhang, Y. Zheng, Y. Lv, F. Li, L. Su, Y. Qin, W. Zeng, Melatonin protects the mouse testis against heat-induced damage, *Molecular Human Reproduction*, 26 **2020** 65-79.
112. Y. Guo, H. Chen, Q.-J. Wang, X. Qi, Q. Li, W. Fu, J. Huang, C.-Y. Yao, Z.-Y. Liu, M.-Z. Wang, Prolonged melatonin treatment promote testicular recovery by enhancing RAC1-mediated apoptotic cell clearance and cell junction-dependent spermatogensis after heat stress, *Theriogenology*, 162 **2021** 22-31.
113. D. Kang, D. Kim, Antioxidant effect of Lonicera Caerulea on heat stress-treated male mice, *Journal of Animal Reproduction and Biotechnology*, 36 **2021** 220-229.
114. G.C. Xavier, P.C. Soares, V.A. da Silva Junior, S.M. de Torres, A.C.M. Maymone, R.N. de Morais, C.S. Moura, M.M.P. Guerra, Effect of dietary selenium and vitamin e supplementation on testicular morphology and serum testosterone concentration in goats following scrotal insulation, *Acta Scientiae Veterinariae*, 44 **2016** 1-8.
115. S.T. Peña Jr, B. Gummow, A.J. Parker, D.B. Paris, Antioxidant supplementation mitigates DNA damage in boar (Sus scrofa domesticus) spermatozoa induced by tropical summer, *PLoS One*, 14 **2019** e0216143.
116. D. Fio, O. Olalekan, O. Azu, I. Okoko, L-arginine augments oxidative stress in cryptorchid testes of adult Sprague-Dawley rats, *JMMS*, 2 **2011** 777-782.
117. D. Kokubu, R. Ooba, Y. Abe, H. Ishizaki, S. Yoshida, A. Asano, S.-I. Kashiwabara, H. Miyazaki, Angelica keiskei (Ashitaba) powder and its functional compound xanthoangelol prevent heat stress-induced impairment in sperm density and quality in mouse testes, *Journal of Reproduction and Development*, 65 **2019** 139-146.
118. A. Kumagai, H. Kodama, J. Kumagai, J. Fukuda, K. Kawamura, H. Tanikawa, N. Sato, T. Tanaka, Xanthine oxidase inhibitors suppress testicular germ cell apoptosis induced by experimental cryptorchidism, *Molecular Human Reproduction*, 8 **2002** 118-123.
119. Y. Cao, Y. Li, Z. Li, F. Wang, C. Li, Dietary zinc may attenuate heat-induced testicular oxidative stress in mice via up-regulation of Cu-Zn SOD, *Genetics and Molecular Research*, 14 **2015** 16616-16626.
120. N. Ghasemi, H. Babaei, S. Azizallahi, A. Kheradmand, Effect of long-term administration of zinc after scrotal heating on mice spermatozoa and subsequent offspring quality, *Andrologia*, 41 **2009** 222-228.