JOURNAL OF VETERINARY AND APPLIED SCIENCES
VOLUME 14, ISSUE 2: 799 – 808 (2024)
Published by: Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria
ISSN: 2315-6856; e-ISSN: 2636-5553; Website: www.jvasonline.com

Effects of Sustanon-250 administered during pregnancy on haematological parameters, serum levels of pituitary-gonadal hormones and fertility indices of pregnant female albino rats

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Abstract

Sustanon-250 is an anabolic androgenic steroid used for clinical therapy, but its effects on female reproductive physiology have not been fully investigated. This study evaluated the effects of sustanon-250 administered during pregnancy on the haematological parameters, serum levels of pituitary-gonadal hormones and fertility indices of female albino rats. Fifteen pregnant female albino rats randomly assigned into three groups (A – C) of five rats each were used for the study. Group A rats (Untreated Control) were given subcutaneous injections of 5 ml/kg body weight (BW) of distilled water as placebo, while Group B and C rats were given subcutaneous injections of 3 mg/kg BW of sustanon-250 and 10 mg/kg BW of sustanon-250, respectively. Treatments were done on gestation days 1, 8 and 15, for all groups. On day 21 of gestation, haematological profile, serum levels of pituitary-gonadal hormones and fertility indices were assessed. Results showed that sustanon-250 administration at 10 mg/kg BW led to a significantly lower ($p < 0.05$) body weight gain (BWG) on day 8 and 15 of gestation, while administration of both 3 mg/kg and 10 mg/kg of sustanon-250 led to a dose-dependent significantly lower ($p < 0.05$) BWG on day 21 of gestation. There was no significant difference ($p > 0.05$) in the mean corpora lutea number (CLN) between the Untreated Control group and group treated with 3 mg/kg of sustanon-250, but there was no pregnancy or corpus luteum of pregnancy established in the rats given 10 mg/kg of sustanon-250. The mean number of implantation sites (IM) was significantly ($p < 0.05$) lower, while serum progesterone level, mean resorbed embryo number (REN) and early embryonic death (EED) were significantly higher ($p < 0.05$) in the group given 3 mg/kg of sustanon-250 when compared with the Untreated Control group. The serum progesterone level of rats treated with 10 mg/kg of sustanon-250 was significantly ($p < 0.05$) lower when compared with that of the Untreated Control group and the group that received 3 mg/kg of sustanon-250. The group given Sustanon-250 administration at 10 mg/kg body weight had significantly ($p < 0.05$) higher mean FSH, LH, PCV, RBC, Hb, MCV and MCH when compared with the Untreated Control group. Sustanon-250, as used in the study, negatively affected fertility in the pregnant albino rats, but improved the erythrocytic profile.

Keywords: Sustanon-250; Fertility; Pregnancy; Pituitary-Gonadal Hormones; Albino rats.

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Article History: Initial manuscript submission received – August 07, 2024; Final revised form received – December 02, 2024; Accepted for publication – December 05, 2024; Published – December 11, 2024.

Introduction

Sustanon-250 is an anabolic androgenic steroid with multiple clinical therapeutic applications (Weber *et al*., 2022). It is composed of four different testosterone esters (testosterone propionate, testosterone phenylprpropionate, testosterone isocaproate and testosterone decanoate), which provide a continuous release of testosterone into the blood to produce a stable testosterone level for a long period of time, extending from 3 – 4 weeks (Harvey *et al*., 2006).

Androgens play an essential role in spermatogenesis and development of secondary sexual characteristics (Oduwole *et al*., 2018). They are primarily synthesized from cholesterol in the process of steroidogenesis, and are produced by the Leydig cells of the testicular interstitium and theca cells (theca interna) of growing ovarian follicles (Miller & Auchus, 2011). Indirectly, androgens regulate reproductive functions through aromatization to oestrogen. Androgen receptors (ARs) have been widely located in the nucleus of female reproductive tissues, namely granulosa cells, theca cells, endometrium and placenta; suggesting a significant role in the physiology of female animals (Gervásio *et al*, 2014; Lissaman *et al*, 2023). They facilitate transition of ovarian follicles from the primordial to the pre-antral stage by amplifying the actions of Insulin-like Growth factor 1 (IGF-1) (Gervásio *et al*, 2014). They also masculinize or defeminize urogenital tract development in female mammals (Abbott *et al*, 2005).

Ovulatory dysfunction usually induced by androgen excess occurs shortly after parturition, around the peri-natal period in rats and mice from embryonic day 18 until post-natal day 6 – 10 (Abbott *et al*, 2005). It has been documented that androgen-induced de-feminization of sexual behaviour usually depends on neuronal conversion of androgen to estrogen metabolites. Such effects of aromatization are particularly evident in females of altricial species exposed to androgen peri-natally (Wallen & Baum, 2002).

Accidental and deliberate ingestion of androgens from synthetic and natural sources is on the increase among female animals and humans (Kanayama & Pope, 2012). However, contrary to the well reported roles of anabolic androgenic steroids on male reproductive function, there is scanty information on their effects on reproductive physiology and fertility of females. The present study investigated the effects of sustanon-250 administered during pregnancy on the haematological parameters, serum levels of pituitary-gonadal endocrine secretions and fertility indices of pregnant female albino rats.

Materials and Methods

Drug used: Sustanon-250 (N.V Organon, London), a synthetic testosterone which contains four testosterone esters (testosterone propionate, testosterone phenylpropionate, isocarproate and testosterone decanoate) was used for the study. Each ampoule contained 1 ml of oily solution of Sustanon-250.

Experimental animals: A total of 15 sexually matured female albino rats (13 – 15 weeks of age) procured from the Department of Veterinary Anatomy Laboratory Animal House, University of Nigeria, Nsukka were used for the study. They were kept in clean metal cages at room temperature of 27 – 32°C at the Laboratory Animal Unit of Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for two weeks before the commencement of the experiment. The rats were fed *ad libitum* on a standard commercial grower feed with 15 % crude protein (Vital®, Grand Cereals and Oil Mills Ltd) and clean water. They were maintained under a cycle of 12 hours light and 12 hours of darkness daily throughout the period of experiment. The research protocol

was approved by the Departmental Research Committee and standard guidelines for use of laboratory animals for experimental purposes strictly adhered to (NRC, 1996).

Experimental Design: Each female rat was successively paired with a male rat. Successful mating was confirmed by the presence of a copulatory (vaginal) plug on the floor of the cage and/or microscopic detection of sperm cells in fresh vaginal smear. The day copulatory plug and/or sperm cells were found was designated day 1 of gestation. Thereafter, simple random sampling method was adopted to assign the fifteen (15) female rats into three groups (Groups A, B and C) of five (5) rats each. The rats were weighed on days 1, 8, 15 and 21 of gestation. Each of the rat groups were treated with the following on days 1, 8 and 15: Group A – 5 ml/kg body weight of distilled water; Group B – 3 mg/kg body weight of sustanon-250, and Group C – 10 mg/kg body weight of sustanon-250. On day 21 of gestation, blood samples were collected for haematology and hormonal assays and laparotomy was conducted to assess the target reproduction parameters.

Haematology: Blood samples for haematology were collected from the albino rats following the orbital technique. The blood samples were collected with the aid of capillary tubes from the retro bulbar plexus of the medial canthus of the eyes of the rats into EDTA-contained plastic sample bottles for haematology. The packed cell volume (PCV) was determined by the haematocrit method, while the red blood cell and total white blood cell counts were done by the haemocytometer method (Thrall & Weiser, 2002). Haemoglobin concentration was determined by the cyanomethaemoglobin method while the differential white blood cell counts were done on Romanowsky stained thin blood smears (Thrall & Weiser, 2002).

Assay for serum levels of female pituitary-gonadal hormones: Two millilitres of blood samples were collected into sterile plain sample bottles (without EDTA) from retro-orbital plexus (medial canthus) of the rats of each group at the end of gestation. The blood samples were allowed to clot and were later centrifuged at 1500 rpm for 10 minutes to separate the sera from the clot. The clear supernatant sera were aspirated into properly labeled vials using a pipette and used for the determination of serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone. Commercially available microplate enzyme-linked immunoassay (ELISA) test kits (Monobind Inc., Lake Forest, USA) were used for the assay.

Determination of Fertility Indices: On day 21 of gestation, laparotomy was performed on the pregnant rats under anaesthesia achieved with simultaneous injection of 5 mg/kg xylazine and 50 mg/kg ketamine. The uterine horns were exteriorized and incised at the greater curvature in order to harvest the fetuses. The percentage of pregnant females (PPF), corpora luteal number (CLN), live foetal number (LFN,) as well as foetal weights (FW), foetal crown-rump length (FCRL), number of implantation sites (IS), and resorbed embryo number (REN) were determined, and fertility index (FI) was calculated using the formula, FI = (LFN × FCRL × PPF) / CLN, as previously prescribed (Mbegbu *et al*., 2017; Nnamonu *et al*., 2020).

Statistical analysis: Data obtained were subjected to one-way analysis of variance (ANOVA) and the means separated using Duncan's new multiple range test. Differences in means less than probability values of 0.05 were considered significant. The computer software, statistical package for social sciences (SPSS) version 21 was used for the statistical analyses. The results were presented as means $\pm$ standard errors of the means.

Results

Effect of sustanon-250 administration on body weight: Figure 1 shows the body weight of the rats during the period of gestation measured on days 1, 8, 15 and 21. There was no significant difference ($P > 0.05$) in the body weight of all the rat groups on day 1 of gestation. However, the mean body weights of the rats given 10 mg/kg sustanon-250 was significantly ($p < 0.05$) lower than those of the other two groups on days 8 and 15 of gestation. On day 21 of gestation, sustanon-250 administration led to a dose-dependent significantly ($p < 0.05$) lower body weight in the Group B and C rats when compared with the Group A untreated control rats (Figure 1).

Haematological profile following sustanon-250 administration: The effect of sustanon-250 administration on the haematological profile is presented in Table 1. The rats groups given 3mg/kg and 10mg/kg body weights of sustanon-250 had a significantly ($p < 0.05$) higher mean packed cell volume (PCV), red blood cell count (RBC) and mean haemoglobin concentration (HB) when compared with the untreated control group. The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) of the group that was treated with 3mg/kg body weight of sustanon-250 did not significantly vary ($p > 0.05$) from that of the untreated control group, but that of the rat group treated with 10mg/kg body weight of sustanon-250 was significantly ($p < 0.05$) higher than that of the untreated control group. Sustanon-250 administration at both 3mg/kg and 10mg/kg body weights led to no significant difference ($p > 0.05$) in the mean corpuscular haemoglobin concentration (MCHC) and the mean total white blood cell count (WBC) of all the rats groups.

Effects of sustanon-250 on pituitary-gonadal hormones (FSH, LH and progesterone): The effects of sustanon-250 administration on serum levels of some pituitary-gonadal hormones is presented in Figure 2. The rat group treated with 10 mg/kg body weight of sustanon-250 had a significantly ($p < 0.05$) higher serum level of FSH and LH and a significantly lower serum level of progesterone when compared to the other two rat groups (untreated control group and the group treated with 3 mg/kg sustanon-250) [Figure 2].

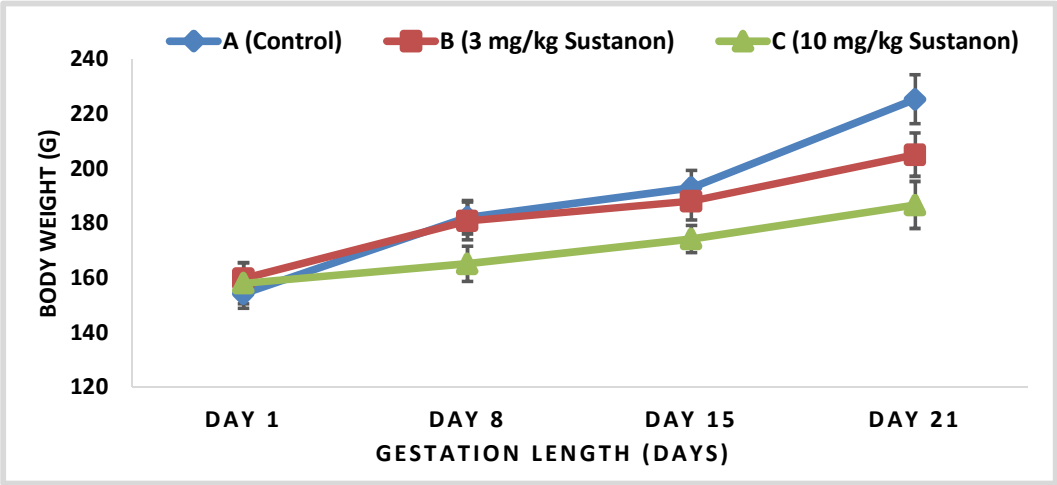

Figure 1: Mean body weights of rat groups treated with varied doses of sustanon-250 compared with an untreated control group. [Group A – 5 ml/kg of distilled water (untreated control); Group B – 3 mg/kg of sustanon-250; Group C – 10 mg/kg of sustanon-250].

Table 1. The haematological profile of rat groups treated with varied doses of sustanon-250, compared with an untreated control group.

Haematological parameters	Mean ± SE of the parameters		
	Group A (Untreated Control)	Group B (3 mg/kg Sustanon-250)	Group B (10 mg/kg Sustanon-250)
PCV (%)	41.40 ± 2.27 [a]	50.20 ± 4.95 [b]	47.20 ± 1.39 [b]
RBC count (×10^6/µl)	10.90 ± 0.40 [a]	13.00 ± 1.40 [b]	12.70 ± 0.70 [b]
HB (g/dl)	14.78 ± 1.63 [a]	19.86 ± 1.42 [b]	17.88 ± 0.95 [b]
MCV (fl)	38.20 ± 2. 20 [a]	38.60 ± 4. 20 [ab]	45.00 ± 3.60 [b]
MCH (pg)	13. 80 ± 1.60 [a]	15.40 ± 1.70 [ab]	17.60 ± 1.90 [b]
MCHC (g/dl)	35.80 ± 2.83	40.28 ± 2.60	37.86 ± 1.57
Total WBC counts (10^3/µl)	7.44 ± 0.71	6.84 ± 0.34	6.68 ± 0.65

[a, b] Different superscripts in a row indicate significant difference in the means ($p < 0.05$)

[PCV – packed cell volume; RBC – red blood cell; HB – haemoglobin concentration; MCV – mean coprpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean copuscular haemoglobin concentration; WBC – white blood cell]

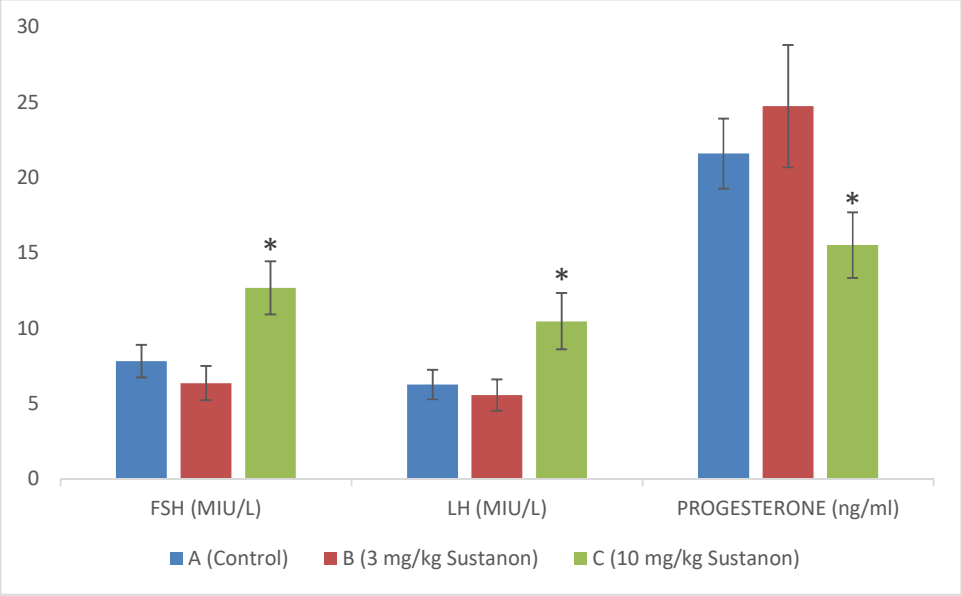

Figure 2. Mean serum levels of follicle stimulating hormone (FSH), luteinizing hormone (LH) and progesterone in pregnant rats treated with sustanon-250, compared to untreated controls.

* Asterisk indicate a mean value that is significantly ($p < 0.05$) different from others.

Table 2. Fertility parameters of pregnant rat groups treated with varied doses of sustanon-250, compared with an untreated control group.

Parameters	Mean ± SE of the parameters (where appropriate)		
	Group A (Untreated Control)	Group B (3 mg/kg Sustanon-250)	Group B (10 mg/kg Sustanon-250)
Percentage of pregnant females (%)	80%	40%	0%
Number of live foetuses	6.85 ± 0.71 [a]	5.68 ± 0.37 [b]	0.00 ± 0.00 [c]
Number of corpora lutea	7.75 ± 1.18 [a]	7.50 ± 0.65 [a]	0.00 ± 0.00 [b]
Number of implantation sites	7.50 ± 1.32 [a]	5.73 ± 0.41 [b]	0.00 ± 0.00 [c]
Number of resorbed embryos	0.50 ± 0.29 [a]	2.25 ± 0.48 [b]	0.00 ± 0.00 [c]
Number of embryos that died early	0.30 ± 0.25 [a]	2.18 ± 0.36 [b]	0.00 ± 0.00 [a]
Foetal crown rump length	4.04 ± 0.05 [a]	3.98 ± 0.06 [a]	0.00 ± 0.00 [b]
Fertility index	265. 23	107.83	0.00

[a, b] Different superscripts in a column indicate significant differences in the means ($p < 0.05$)

Effects of sustanon-250 administration on female fertility parameters: The results of assessment of fertility indices is presented in Table 2. The mean percentages of pregnant females (PPF) in the groups were 80% for the untreated control group, 40% for the group treated with 3 mg/kg sustanon-250, and 0% for the group treated with 10 mg/kg sustanon-250. There was no significant difference ($p > 0.05$) in the mean corpora lutea number (CLN) between control group and group that received 3mg/kg body weight of sustanon-250. No corpora lutea of pregnancy was found on the ovaries of rats in the group that received 10mg/kg body weight of sustanon-250. The mean number of implantation sites (IS) in the group that received 3mg/kg body weight of sustanon-250 was significantly lower ($p < 0.05$) when compared with the untreated control group. No implantation site was observed in the group that was given 10mg/kg body weight of sustanon-250. The mean number of resorbed embryo and early embryonic death (EED) of the group that was given 3mg/kg body weight of sustanon-250 was significantly ($p < 0.05$) higher when compared with that of the untreated control group. The group that was given 10mg/kg of sustanon-250 showed no pregnancy; therefore, neither embryo resorption nor death was observed. There was no significant difference ($p > 0.05$) between the group given 3 mg/kg sustaton-250 and the untreated control group in the foetal crown rump length, but the fertility index of the group treated with 3 mg/kg sustanon-250 was lower than that of the untreated control group (less than half of that of the untreated control group), while that of the group treated with 10 mg/kg sustanon-250 was 0 (Table 2).

Discussion

The dose-dependent lower body weight observed with sustanon-250 treatment could be largely attributed to the recorded reduction

in conception/implantation rates and pregnancy failure. When conception is achieved and pregnancy established, the growth and development occurring in the embryos/fetuses contribute to the weights of the dams (Aguilera, *et al.,* 2022). Androgens are classified among fat reducing hormones, and the fat-splitting property of testosterone (testosterone and dehydroepiandrosterone) has been demonstrated (Sebo & Rodeheffer, 2021). In particular testosterone inhibits lipid uptake and lipoprotein-lipase activity in adipocytes and decrease of adipocyte leptin production. It also inhibits differentiation of adipocyte precursor cells (De Pergola, 2000). On the other hand, DHEA stimulate resting metabolic rate and lipid oxidation. It has been found that fat cell exposure to testosterone induced an increase in the number of androgen receptors in a dose dependent way (De Pergola, 2000). The lowered body weights invariably resulted from the inherent fat-reducing ability of androgens.

Embryo losses in rodents manifest as early resorption, late embryo death or foetal death (Ronald, 2012). From the assessed fertility parameters, the percentage of pregnant females in the control group was 80% while treatment with 3mg/kg and 10mg/kg body weight of sustanon-250 elicited 40% and 0% conception rates respectively. It is possible that sustanon-250 hindered normal folliculogenesis and altered ovarian steroidogenic activity. The lack of significant difference in the number of corpora lutea between the control group rats and rats treated with 3mg/kg body weight of sustanon-250 proved that 3 mg/kg body weight of sustanon-250 did not only prevent implantation, but caused embryo resorption/death. Furthermore, the reported contractile effect of sustanon-250 (Shigehara *et al,* 2022) could distort fertilization process and implantation, hence the observed lower number of implantation sites and increased embryo resorption in sustanon-treated groups. Anti-fertility property of testosterone in females has been attributed to its vasoconstrictory effect of testosterone in the pregnant rat (Iliescu and Reckelhoff, 2006). Chinnathambi *et al* (2014) observed that elevated testosterone levels increased maternal blood pressure and decreased blood flow to various tissues including the placenta. In a similar experiment, it has been suggested that foetal loss possibly occurred at the conception phase or after implantation of non-viable fetus as evidenced by the marked embryo resorption (Ejembi, 2016).

Previous reports showed that Sustanon-250 adversely affect secretion of ovarian steroids (estradiol and progesterone) and gonadotropins (LH and FSH), due to damage of ovarian tissues and atrophy (Nehaya and Mohammed, 2014). This reportedly leads to granulosa cell death and follicular atresia with consequent failure in corpus luteum formation and progesterone production needed for sustaining pregnancy. This could explain the observed low concentrations of progesterone in sustanon-treated groups in the present research.

Administration of sustanon-250 at 10 mg/kg body weight led to significantly higher serum levels of FSH and LH when compared with the control group and group that received 3mg/kg. This higher serum level of gonadotropins could be associated with conception failure and absence of corpora lutea of pregnancy in this group. Corpora lutea of pregnancy secretes progesterone that is needed to maintain pregnancy (Cable and Grider, 2023). On the other hand, progesterone regulates LH and FSH secretion by negative feedback mechanism (Nedresky and Singh, 2022). This also agrees with the findings by Nehaya and Mohammed (2014), where sustanon-250 altered the secretion of ovarian steroids and gonadotropins due to damage on ovarian tissue and atrophy.

This present study showed that administration of sustanon-250 led to significantly higher erythrocytic parameters and no significant effect on total white blood cell counts. The higher packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) suggests that sustanon-250 administration favoured erythropoiesis, which implies adequate tissue oxygenation and better cellular function of the treated rats. This is in consonance with the earlier findings that testosterone increased haemoglobin concentrations, PCV and RBC, and improved recovery from haemorrhage in hypogonadal males (Olagbade and Ladoke, 2002). Similarly, Bachman *et al* (2013) posited that testosterone induced increases in haemoglobin concentration and hematocrit are a result of stimulation of erythropoietin and reduction in ferritin and hepcidin concentrations.

In conclusion, data from this experiment suggest that sustanon-250 administration as used in the study exerted anti-fertility effects and led to poor pregnancy outcome, though it improved erythrocytic parameters in pregnant albino rats.

Conflict of interest

The authors therefore declared no conflict of interest.

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